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THE PATHOLOGY OF TRENCH FOOT *

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Injuries due to cold have been known for many years, but the study of such lesions always gains new impetus in wartime. In addition to frostbite and trench foot, which have been investigated in the past, new varieties of thermal gangrene in the form of high altitude frostbite of aviators [1, 2] and immersion foot of seamen [3] have made their appearance in the present war. Through the cooperation of a number of medical officers, material from 14 cases (Table I) of trench foot, which occurred in two different theaters of operation, has been received at the Army Institute of Pathology. The alertness of several contributors † who realized the great importance of studying the early phases of the disease process made it possible to obtain tissue from soldiers who died of other causes while suffering from an incipient stage of trench foot. Study of this invaluable material in conjunction with tissue illustrating the intermediate phases and late sequelae has enabled a reasonable picture of the pathogenesis to be reconstructed.

CLINICAL DATA

Almost all of the patients were in their twenties; the oldest was 34. There was one Arab, one Mexican, and one Frenchman; the others were all white Americans from various parts of the Union (Michigan, Maryland, Mississippi, and New York were among the states represented). It is unfortunate that exact data about the weather conditions during the period of exposure are not available in all cases. In some instances the only information submitted was that the patient had "trench foot" or "frostbite"; in others it was stated that the soldiers had been exposed to cold water or to snow.

For one group of patients, cases 8 to 13, all of whom suffered injury during the invasion of Attu, accurate data compiled on nearby ships

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and airbases were obtained through the cooperation of the Weather Division, Office, Assistant Chief of Air Staff, Operations, Commitments and Requirements. These records indicate that the freezing point was not reached throughout the week during which exposure took place. The temperature on board ship, presumably within firing range offshore, was 33°F. at its lowest, but usually ranged between 38° and 41°F. The dew point was rarely more than 2 or 3 degrees lower than the temperature, and the relative humidity was often 96 per cent or higher. The wind velocity was less than 10 knots about half the time and lower than 30 knots for most of the remainder of the period. The data recorded at the nearby bases on shore indicate even milder weather. During the first few days of the operation the temperature ranged between 38° and 42°F.; it later fell, 33°F. being the lowest point reached. Humidity was high, but the wind velocity, which was lower than on shipboard, never exceeded 18, and was usually less than 14 miles per hour. The weather conditions on Attu itself were perhaps somewhat more severe in view of the altitude and the mountainous snow-covered terrain, but they never attained so-called "true frostbite" severity. The patients stated that the temperature ranged from slightly above to slightly below freezing. In the coldest areas the ice and snow on the ground melted during the day and froze again at night. Thin skins of ice covered pools and wet areas in the morning. Some of the men's feet became wet during the landing and remained wet for as long as 6 days. The soldiers all kept their boots on for several days.

In 3 cases (nos. 1 to 3), henceforth referred to as "early," material was secured at autopsy from patients who died 7 to 10 days after the onset of exposure, from conditions other than trench foot. Their feet had first become cold and numb, but the pulses remained palpable, and there was no swelling. Edema, increased warmth, and cyanosis appeared soon after rescue and were followed by the development of bullae, hemorrhage, and anesthesia; these lesions were present at the time of death. The blood-tinged fluid from one bulla had a specific gravity of 1.028 and contained 6.8 gm. of protein per 100 cc. and a few cells.

In the remaining 11 "late" cases (nos. 4 to 14) amputation for gangrene was performed 1 to 5 months after exposure. Some patients lost only the toes and others had midtarsal amputations, but most of the operations were low or mid-leg resections. The lesions were usually bilateral and symmetric, but occasionally asymmetric involvement resulted in unilateral amputations.

The process in these late cases had also begun with a "white numb"

TABLE I

Fourteen Cases of Trench Foot

Case	Type of exposure	Duration	Interval from exposure to examination	Type of reaction	Complications
1. Autopsy	"Frostbite"	Not known	7 days	Edema, hemorrhage, cyanosis, bullae	Gunshot wound of head, hemiplegia
2. Autopsy	Cold water	8 hours	10 days	Edema, hemorrhage, cyanosis, bullae	Gunshot wound of leg, fracture, plaster casts
3. Autopsy	Cold and snow	26 hours	10 days	Edema, hemorrhage, cyanosis, bullae	Fracture, gunshot wound of leg, cold injury to hands, hemoglobinuric nephrosis, uremia
4. Amputation	"Trench foot"	Not known	30 days	Gangrene	Infection
5. Amputation	"Trench foot"	4 days	32 days	Gangrene	Infection (*Staphylococcus aureus*)
6. Amputation	Wet and cold	7 days	40 days	Gangrene	None recorded
7. Amputation	"Trench foot"	Not known	42 days	Gangrene	None recorded
8. Amputation	Wet and cold, not freezing	8 days	50 days	Gangrene	Gunshot wound of shoulder, infection (*Staphylococcus aureus; Clostridium welchii*, no gas)
9. Amputation	Wet and cold, not freezing	4 days	50 days	Gangrene	Infection (*Staphylococcus aureus; Streptococcus haemolyticus; Clostridium welchii*, no gas)
10. Amputation	Wet and cold, not freezing	6 days	70 days	Gangrene	Infection (*Clostridium welchii*, gas), cold injury to fingers
11. Amputation	Wet and cold, not freezing	6 days	77 days	Gangrene	Infection (*Streptococcus haemolyticus; Staphylococcus aureus; Bacillus pyocyaneus*)
12. Amputation	Wet and cold, not freezing	Not known	121 days	Gangrene	None recorded
13. Amputation	Wet and cold, not freezing	6 days	143 days	Gangrene	Infection, cold injury to fingers
14. Amputation	"Trench foot"	Not known	Not known	Gangrene	None recorded

stage and evolved through a hyperemic phase into gangrene. At the beginning of exposure the legs were painful for 12 to 24 hours before they became numb or swollen. In one case the feet stayed numb and the soldier "walked on wood" for 6 days, but less than an hour after removal of his boots the feet were swollen and blue, and in 3 or 4 days the toes were already stiff and dry. Although, in one of the early cases, gangrene of the tips of the toes was evident on the seventh day, in most instances 3 or 4 weeks elapsed before frank gangrene set in. In the stage of gangrene the frequent inability to feel the dorsal pedal pulses despite the presence of the posterior tibial pulsations may have been due to local swelling. In the one case in which both the dorsal pedal and the posterior tibial pulses were felt, gangrene extended only to the midtarsal region. The pulses varied in strength from day to day, and there was some improvement after sympathetic block.[4]

As a result of crawling, some patients had lesions on the knees; others had involvement of the hands. Occasionally hyperextension of the toes and a foot deformity resembling Volkmann's contracture developed. *Staphylococcus aureus, Streptococcus haemolyticus* and *Clostridium welchii* were among the organisms cultured from secondarily infected lesions; in one case there was inguinal adenopathy.

Cases 8 to 13 were included in Patterson's [4] group. His report gives additional clinical details and includes photographs of some lesions.

Gross Pathologic Findings

The legs in the early cases showed swelling, blebs, bullae, hemorrhage, and cyanosis (Fig. 1). The skin peeled off easily, and the surface was red and injected. The edematous subcutaneous tissues were gelatinous, and the fat appeared abnormal. Usually the damaged portion of the foot was sharply demarcated, at a level slightly below the malleoli, from the proximal normal tissues; moreover, the severely injured superficial layers were also set off from the less involved deep tissues. The subcutaneous veins were dilated, and in one case the dilatation was seen to end abruptly at the demarcation zone. The engorged vascular plexuses showed as a network of tortuous varicosities in the subcutaneous tissues.

In case 2 there were a Pott's fracture about 3 inches above the left ankle, a small penetrating wound on the dorsum of the right foot, and a comminuted fracture of the os calcis which was believed to have interfered with the circulation. Tight casts had also been applied to the legs. A compound comminuted fracture of the lower third of the right femur and penetrating wounds in the posterolateral aspect of the left

thigh complicated the picture in case 3. (A section of the right femoral vein revealed only slight disruption of the wall, scanty cellular infiltration, and a small mural thrombus, while the femoral artery was normal.) In both these cases the possibility must be considered that some of the changes in the tissues resulted from the wounds and fractures, but local damage was minimal in contrast to the extensive injury attributed to cold. In case 1 there were no traumatic lesions of the extremities, but the patient had a severe head wound. Although the effect of the resultant hemiplegia on the peripheral vasomotor disturbances cannot be ignored, the severity of the reaction and the involvement of both feet suggest that the damage to the central system played no significant part in the production of the lesions of the legs. In all 3 cases the lesions ascribed to cold exhibited a basic similarity.

In 3 of the late cases gangrene was restricted to the toes (Fig. 2), but usually it involved the whole foot, almost to the level of the malleoli (Fig. 3). The skin was black and dried, and the tissues appeared desiccated and mummified. In some instances the entire sole, or "sandal area," was blackened, while in others the midportion was spared but the heel was involved. Above the ulcerated zone, 1 to 6 cm. in width, which formed the line of demarcation, the tissues were swollen, cyanotic, and purple-yellow. The ulceration exposed tendons and bone. The soft tissues beneath the black surface membrane were necrotic, and the muscles were either red and putty-like or gray and watery. Although purulent exudate was abundant, foul liquefaction and gas bubbles were present in only one case. The subcutaneous tissues which were not necrotic were gelatinous and edematous, and yellow and orange "congealed" foci were observed in the subcutaneous fat. Thrombosis or thickening of the dorsal pedal, medial plantar, and lateral plantar arteries and other vessels was noted in several instances. Some vessels and nerves frayed out and disintegrated as they passed into the areas of gangrene. Complicating infection resulted in such a variety of inflammatory reactions that it was often difficult to disentangle the lesions due to cold from those produced by the secondary invaders.

Roentgenologic Findings

Roentgenograms of the bones of the foot in an early case and in the 30-day and 70-day cases seemed normal. In one 50-day case both early osseous atrophy and, near some joints, resorption which appeared secondary to infection of the soft tissues were evident. Slight absorption and formation of new bone at the amputated metatarsal stumps and considerable atrophy of disuse were observed in the 143-day case.

Microscopic Findings

Skin and Subcutaneous Tissues

Epidermis. In the early cases there were numerous vacuolated edematous cells in the malpighian layer of the intact skin. Foci of intercellular edema and small vesicles containing fluid and a few leukocytes were noted. Sections through hemorrhagic bullae showed either cleavage in the malpighian layer or stripping off of the basal layer from the papillae. In some places the superficial layers were necrotic. In late cases there was surprisingly little epithelial proliferation at the edges of ulcerated areas. Occasionally the epidermis was thinned out over inflamed and thickened subcutaneous tissue and had lost its pegs, but in other regions it was thickened. In places increased amounts of keratinized material were retained on the surface and in the follicles. In the mummified areas the epidermis was shrunken and desiccated and the clearly defined granular layer stood out prominently. There were bacterial masses on the stratum lucidum, which formed the surface layer. The vacuolation of the cells of the malpighian layer was still evident.

Appendages. The sweat glands in the early cases showed cellular degeneration, and the coils and the ducts were often lined by a fibrinoid layer of homogenized hyalinized cells, pyknotic nuclei, and chromatin débris. The vascular plexus surrounding the glands stood out as a conglomeration of dilated and engorged channels, some of which were thrombosed. A moderate infiltration of leukocytes was present about the glands and vessels. In mummified areas in the late cases the appendages appeared as clumped masses lying in hyaline necrotic collagen. Although the hair follicles were somewhat better preserved, the sebaceous glands were shrunken. In less damaged areas mucinous change in the periglandular connective tissue and perifollicular fibrosis were evident.

Dermis and Subcutaneous Tissue. In early cases the collagen of the dermis was occasionally degenerate or necrotic; the scanty shredded elastica stained poorly. The distal regions showed some of the desiccation so characteristic of the mummified tissue in the late cases. The dermis, in general, was free from cellular exudate, except in the immediate vicinity of congested vessels about the appendages. The stripped papillae, which were shrunken and basophilic, contained nuclear débris and a few leukocytes about the capillaries. Strikingly distended and tortuous papillary loops (Fig. 4) were encountered. Ulcerated surfaces were lined by inflamed granulation tissue or a fibrinoid necrotic membrane. Edematous papillary processes underlay vesiculated epidermis. Agglutinated red cell masses plugged some vessels in the subpapillary

plexus. In the late cases heavy infiltrations of leukocytes, eosinophils, and round cells were evident in areas of cellulitis, but fibrin was not abundant. The collagen was necrotic in areas of gangrene; in the mummified areas the dead fibers, which were shrunken and desiccated, had no nuclei or chromatin débris, were packed together in compact, slightly basophilic bands, and resembled the collagen of burned tissue (Fig. 5). Despite the presence of bacterial colonies in the dead connective tissue, no cellular reaction was evident. Clumped red cell masses filled the dilated blood vessels. Near the zone of demarcation the loose areolar portions of the connective tissue showed edema and mucinous degeneration. In other areas there were dense keloid-like collagenization and scarring, and degenerated elastica (Fig. 6) formed thickened, knotted strands and fragmented segments. In some foci the collagen was broken into irregular granules but stained normally. Cellular infiltration was evident throughout; a few aggregations of cells, which included eosinophils and stem cells, resembled hematopoietic foci, and in one case a marked plasma cell reaction was noted. Increased vascularity, congestion, and hemorrhage characterized many regions, and occasional large collections of hemosiderin-laden macrophages were encountered. Inflamed granulation tissue was the rule in ulcerated areas.

Fat

In early cases infiltration by leukocytes was pronounced in the deeper subcutaneous adipose tissue and in the fat around the appendages even when the overlying layers were not involved. The adventitial cells of the prominent capillaries and smaller vessels in the fat lobules had proliferated. The interlobular fibrous septa showed edema and leukocytic infiltration. There was some fibrinous exudate in the deeper tissues.

Changes in the fat, which were marked in the late cases, were already pronounced in the 32-day case. Foam cells laden with finely divided fat (Figs. 7 and 8) had diffusely infiltrated the fat lobules; for the most part they were smaller than the original fat cells, although an occasional multinucleated form of considerable size was encountered. Rare accumulations of giant cells of the foreign body and Touton types were observed in otherwise unaltered fat. Actual fat necrosis with soap formation was rare; it occurred only in areas of gangrene in the 143-day case. The many minute and few large oil cysts (Fig. 9), which were lined by a layer of foam cells, contained scattered, free, fat globules. Within the adipose cells the fat was occasionally finely divided in place of being smoothly homogeneous. Neither this change in texture nor the previously mentioned lipoid phagocytosis suggested to

me that a reversion to "glandular," or embryonal, fat had taken place, but some other pathologists who studied the sections expressed the belief that such was the case. In a few fat cells doubly refractile sheaves of fine needle-like crystals were noted. Although some changes were especially marked near areas of gangrene and cellulitis, many foci were present in the subcutaneous panniculus, even above the line of demarcation.

Fibrous replacement of adipose tissue took place in two ways. In some regions serous atrophy or actual replacement by loose areolar or mucinous connective tissue (Fig. 10) left a collapsed atrophic structure in which the original outline of the fat lobule was still preserved. In others thickening of the interlobular fibrous septa (Fig. 11) had resulted in conspicuous depletion of the adipose tissue component in the subcutaneous layer.

In many areas of gangrene, especially near mummified regions, the adipose tissue was necrotic; the nuclei had disappeared, but unaltered large fat globules persisted in the dead cells. There was often a faint yellow discoloration, as if there were staining by hemoglobin.

Blood Vessels

Marked engorgement of the vascular tree characterized the early cases. The capillaries and small vessels in the papillary loops, the subpapillary plexus, the networks about the sweat glands and appendages, and the subcutaneous plexus in and about the fat lobules were as clearly outlined as in tissue injected for teaching purposes. Many medium-sized vessels were so dilated, rounded, and thin-walled that they seemed to be paralyzed, as in Ricker's "peristasis." * Extravasated red cells surrounded the engorged plexuses. The erythrocytes in the distended papillary vessels stained poorly, and there was hemolysis in the superficial vascular channels in some regions. In the deeper plexus the red cells were discrete and well preserved, although in some hyperemic foci they appeared spherocytic. Occasionally clumped masses of red cells had become homogenized and hyalinized; other aggregations had been transformed into brightly eosinophilic granular material. Fat, possibly derived from the breakdown of stagnating blood, was present in the lumina of the papillary and subpapillary vessels.

Numerous vessels contained agglutinative erythrocytic thrombi (Fig. 12) of the type which form in stagnant rather than in streaming blood. Only a few pure fibrin plugs were noted, and the amount of fibrin in the other type of thrombus was minimal. In one dorsal pedal

* Ricker, G. Sklerose und Hypertonie der innervierten Arterien. J. Springer, Berlin, 1927.

artery, the wall of which was partially necrotic and infiltrated with leukocytes, there was organization of fibrin at the base of a red cell thrombus. A few small subpapillary channels contained masses of finely granular material (Fig. 13) which presumably were composed of platelets. Platelets were included in the red cell thrombi in medium-sized and large vessels and formed skeletal networks (Fig. 14) both in arteries and in veins. In the veins hemorrhage and thrombosis were often especially marked in relation to the valves. Some vessels contained mixed thrombi composed of red cells, platelets, hyaline material, and enmeshed leukocytes. An occasional yellow-green patch suggested hemolysis. The thrombi usually entirely filled the lumina of the involved vessels, but incomplete plugs were encountered, and rarely mural deposits of hyaline material or fibrin encircled a patent central lumen.

Endothelial damage was not a striking feature, although rare, swollen, vacuolated endothelial cells bulged into the lumina of the smaller vessels. Mural hemorrhage (Fig. 15) and inflammation (Fig. 16) of both plugged and patent vessels, but no periangiitis, were observed. The walls contained leukocytes and chromatin débris, and many coarse and fine granular eosinophilic masses of material replaced the sharply staining cytoplasm of the muscle elements.

Many vessels, especially if thrombosed or inflamed, were dilated, but markedly constricted (Fig. 17) large arteries were also encountered. Vasoconstriction was present even in main trunks well above the line of demarcation, but it is difficult to be certain that such vessels were in the contracted state during life.

In the 32-day case transitions (Fig. 18) from the stage of thrombosis seen in the early cases to the picture of endarteritis obliterans were observed. Proliferation of connective tissue and capillaries and the development of a mucinous stroma were observed in relation to the presumably original thrombi, in which the predominance of red cell and platelet agglutinations and the paucity of fibrin were still evident.

As early as 40 days after the original injury almost all thrombi were already organized, and both arteries and veins showed the features of endangiitis obliterans, even in practically normal tissues above the line of demarcation. The degree of endarteritis varied from slight thickening of the intima to obliteration of the lumen. Slightly involved arteries showed subintimal proliferation of cells, often in a mucinous and edematous matrix (Fig. 19). The intimal thickening was frequently eccentric, but sometimes the entire circumference was involved and in extreme cases marked narrowing of the lumen resulted. The lumina of obliterated arteries (Fig. 20) were filled with fibroblasts, round cells, and hemosiderin-laden phagocytes. Usually the central mass contained

a number of discrete channels which had definite muscular walls and resembled arterioles (Fig. 21). Similar recanalization was noted even in small arteries and arterioles. The proliferative reaction was central to the inner elastic membrane, which was usually intact and not reduplicated. Rarely, involvement of the vessel wall resulted in destruction of the elastica (Fig. 22).

The veins were less regularly involved than the arteries, but they showed nodular intimal thickening caused by edema, mucinous degeneration, and increase in cells, collagen, and elastica. Some were obliterated, but others had labyrinthine lumina (Fig. 23), which may have resulted either from recanalization or from polypoid endophlebitic proliferation. The thickened network of tissue which traversed the central cavity occasionally appeared to be based on pre-existing valvular structures. The strands contained intracellular deposits of hemosiderin and even small, muscled, arteriole-like channels (Fig. 24). Although eccentric involvement occurred, the damage was not confined to the side of the vessel directed toward the skin surface.

Even in late cases occasional vessels which were dilated and thrombosed but still unorganized were found. Some showed necrosis, which was never typically fibrinoid, hemorrhage, and cellular infiltration. Mucinous degeneration, vacuolization, and edema often separated the cells and lamellae of the media even in the absence of intimal change. Perivascular fibrosis, proliferation of adventitial cells, and infiltration of round cells and eosinophils were all encountered, particularly in inflamed regions, but no real periarteritis was evident.

In the areas of gangrene and cellulitis many large vessels and their contents were discernible as ghost structures although they were completely necrotic. A few thrombi were observed; one of these was septic and contained cocci. In mummified areas masses of red cells were still visible in the smaller necrotic vessels, which were lying in the midst of homogenized anuclear necrotic collagen. Some contents suggested fused masses of hemoglobin without cellular structure, but in others the individual red cells were clearly defined.

Muscle

In the early cases the muscle showed degeneration, necrosis, and cellulitis but no atrophy. Although engorgement of vessels, which was so striking in the more superficial tissues, was not observed, thrombi were present in small channels. In case 2 (10 days), in which the leg was in a tight cast for a few days, circumscribed foci of necrosis developed, and an actively phagocytic mononuclear reaction was noted.

Many macrophages had surrounded dead fibers and penetrated the endomysial membrane. Little granulomas, composed of such macrophages, remained where fibers had been destroyed. The infarction observed may have been related to compression since it was absent in the 2 other early cases, but well developed encapsulated infarcts (Fig. 25) were noted in case 5, one of the earliest of the late cases (32 days).

Extensive atrophy (Fig. 26) was noted in the late cases as early as 40 days after exposure (case 6). Although fibrils could be identified, the cytoplasm was usually shrunken and homogenized. Cells laden with yellow pigment lay between the atrophic fibers, which were occasionally separated from the endomysial network by spaces containing edema fluid; the interstitial connective tissue had undergone mucinous degeneration. Proliferation of sarcolemmal nuclei was most evident in the less atrophic areas, but only a few true muscle giant cells were observed. The muscle showed necrosis and inflammation in the areas of gangrene and cellulitis. Circumscribed infarct-like foci of necrosis were present in the zone of demarcation and above. Hyaline degeneration or proliferation of sarcolemmal nuclei was encountered in occasional isolated fibers. Many tendon sheaths exhibited severe exudative and proliferative lesions in which masses of fibrin were more prominent than in the regions of inflammation elsewhere in the soft tissues.

Nerves

In the early cases the nerves which traversed regions of inflammation were swollen and edematous. Even away from such areas degeneration, both of axis cylinders and of myelin, was observed. Large fibers were irregularly broken, beaded, and frayed (Figs. 27 and 28). Myelin balls were distributed along the segmented and fragmented nerve sheaths. Demyelinization was especially marked in the distal portions; in case 1, for example, the medial plantar nerve was extensively damaged (Fig. 29), while the posterior tibial trunk was less involved. Although occasionally swollen Schwann cells contained finely granular fat, the pronounced lipoid phagocytosis so evident in the late cases was lacking. The groups of small and nonmyelinated fibers which presumably represented the sympathetic components of the nerves were not significantly altered either in the main trunks or in the small branches (Fig. 30) near blood vessels; only the large myelinated fibers were affected. The small intraneural vessels showed no essential abnormality.

In the late cases the nerves in regions of gangrene and cellulitis were usually badly damaged, but occasionally a trunk traversing a necrotic area was fairly well preserved. The demyelinization (Fig. 31) which

was seen at all levels was more extensive below the zone of demarcation; often only a few clumps or beaded columns of myelin remained along the axis cylinders (Fig. 32).

Between the nerve fibers there were many foam cells (Fig. 33) which contained sudanophilic material in fine droplets, presumably fat from broken down myelin. In case 13 fine crystalloid spicules also were seen in these lipoid phagocytes. Many axis cylinders had disappeared; those still present were irregular and ballooned (Fig. 34). Damage was usually spotty, so that involved and uninjured fibers were often haphazardly intermingled. Some nerve bundles showed edema and separation of the fibers; actual increase of the endoneurial connective tissue elements may have been present in a few. Perineural fibrosis (Fig. 35) with exaggeration of the epineurium and perineurium was observed, and occasional nerve bundles were partially or completely hyalinized. Many small blood vessels in the nerves were thickened. The degeneration and phagocytosis seen in the subcutaneous adipose tissue were also evident in the epineurial fat.

Bone

Bone was available for study in only one early case. A section of the middle phalanx of a toe revealed no significant abnormality. More extensive study was possible in the late cases. Adjacent to regions of cellulitis were areas of osteomyelitis; the inflammatory exudate had occasionally undermined and eroded articular cartilages. Except in such areas there was little evidence of resorption or of osteoclastic activity; sequestration was not noted. Near the zone of demarcation necrosis of bone had occurred, and the osteocytes had disappeared from the lacunae. A sharply defined layer of viable bone surrounded the dead lamellae. In some regions about the necrotic trabeculae numerous osteoblasts were actively laying down new bone (Fig. 36). In places the bone marrow was necrotic or involved in the osteomyelitic process. Elsewhere it showed serous atrophy, fibrosis, hemorrhage, and infiltration of inflammatory cells. There were many foci of lipoid phagocytosis comparable to those seen in the subcutaneous panniculus; occasional small oil cysts were noted. The altered marrow contained an increased number of thin-walled dilated vessels.

Comment

Skin and Subcutaneous Tissues

Early degeneration of the epidermis after exposure to cold has been repeatedly described,[5-7] but Siegmund [8] expressed the belief that the vacuolation of epithelial cells might be an artifact of thawing. Davis and co-workers [1] attributed the vesiculation and formation of bullae to

transudation. During the regeneration of damaged epithelium, syncytial elements and giant cells may appear, and amitotic division has been said to occur.[5, 6] Late atrophy [9] and flattening of the papillae have been noted,[10] and Siegmund also described hyperkeratosis and melanosis of the basal layer. Fuerst reported generalized involvement of the appendages,[6] and Rémy and Therèse [11] observed necrosis, degeneration, and even proliferation of sweat glands. Their reference to hyaline cylinders suggests that they saw the same central layer in the sweat glands which was noted in the early cases of this series. Siegmund mentioned loss of hair follicles and sebaceous glands and the presence of reduced numbers of sweat glands which had swollen basement membranes.

The diffuse sclerosis which may follow cold injury has been attributed to transudation.[9] Siegmund [8] suggested that proliferation of mesenchymal cells and their products resulted from the high protein content of the fluid. Such fibrosis, which Rémy and Therèse [11] first described and termed "lardaceous inflammation," has been held responsible for inelasticity and rigidity of tissues and consequent restriction of movement.[9, 12] Marchand[13] expressed the belief that the general thickening of tissues which followed cold injury might have a protective action. Small vessels are constricted by the thickened matrix,[8, 9, 12] but since telangiectasia also occurs, the resultant picture has many features in common with radiation reaction.[12] Rémy and Therèse emphasized perivascular sclerosis and Siegmund found adventitial proliferation. Hemosiderin-laden macrophages and an increased number of melanophores may be scattered about in the dermis.[8] Degeneration and even disappearance of elastica may occur throughout the connective tissue.

Changes in the subcutaneous fatty panniculus have been described but not stressed, and no special significance has been ascribed to them. Some observers [14, 15] made no mention of damage to adipose tissue although it was evident in their published photographs. Siegmund [8] noted free fat droplets and soap formation in the areas of fat necrosis even in early cases; he also found pulmonary fat emboli. In the early cases of the present series the only changes in the subcutaneous panniculus were engorgement of the vascular bed of the lobules and exudation of fluid and leukocytes. Pulmonary fat emboli were observed in two of the three cases in which autopsy was performed, but fractures were present in both instances. Lipoid phagocytosis was not seen within the first month after exposure, and soap formation was observed only in one late case. The fibrosis and atrophy of the subcutaneous fat, which were so extreme in the late cases, have been pointed out by a number of workers.[7, 8, 10, 11, 16]

Inflammation of adipose tissue and alteration of fat have been de-

scribed in perniosis.[17-19] The rôle of cold in the production of the adiponecrosis of infants and adults [20] is well known. In a case of "cold allergy" studied by Heid and Fromer,[21] panniculitis developed after brief local application of ice; the process began with leukocytic exudation, and its end-result was a picture indistinguishable from that of Weber-Christian disease.[22] Degeneration of myelin is hardly a specific process, but the possibility that its occurrence in the early cases was a direct effect of cold cannot be ignored. The absence of crystallization and fat necrosis in the early cases is evidence against the theory of a special vulnerability of lipoid tissues to cold, despite Smith's report [23] of fat necrosis and pancreatitis following cryotherapy. Although it has not been determined whether the vascular bed of fat tissue is exceptionally susceptible to damage by cold, it is known that these vessels react to low temperatures. It is probably reasonable to consider that the changes in the fat in the late cases were secondary to the vascular involvement, even though thrombotic and obliterative lesions of vessels were not always demonstrable in regions of injured adipose tissue. Rich [24] suggested that the pressure of clothing on tissue devitalized by vascular obstruction may have led to traumatic fat necrosis.

Nerve and Muscle

Degeneration of nerves after experimental exposure to cold has been described frequently,[5, 7, 11, 25, 26] and the neural changes in frostbite, trench foot, and immersion foot [11, 15, 27, 28] have often been emphasized. Involvement is sometimes evident grossly by the edematous and glassy appearance of the nerves. The earliest changes, consisting of stasis, hemorrhage, cellular infiltration, and exudation of protein-rich plasma, which were described by Siegmund,[8] were not encountered in the present series. Blackwood [14] noted only edema of the nerve-muscle "leash" with separation of the fibers in the one early case of immersion foot which he studied, although he and Russell [26] had found wallerian degeneration early in their experimental material. Degeneration of axis cylinders and myelin, which is characteristic of late stages,[8, 14] was already present in the early cases of the current group. It has been generally agreed that the neutral fat which was observed in macrophages or Schwann cells is derived from broken-down myelin. Blackwood described regeneration of nerve fibers and proliferation of Schwann cells in late cases. Panchenko [29] and Siegmund [8] noted retrograde degeneration of ganglion cells. Perineural fibrosis and hyalinization of nerves, which have been reported in late cases, have been held responsible for the pain which occurs in some stages of immersion foot [12] and frostbite.[11]

The spotty nature of the involvement in some nerve trunks suggested to Siegmund [8] that the damaged groups might represent sympathetic fibers. In the early cases of the present series unequivocal neural damage was evident, but it was the large medullated rather than the small nonmyelinated fibers which were injured. Blackwood [14, 94] stated that in the nerves which he studied all except the small myelinated and unmyelinated fibers were affected. The destruction of myelin and lipoid phagocytosis were so striking in the late cases of Siegmund's series and of the present group that damage predominantly to medullated fibers seems indicated. Siegmund found only minimal changes in sympathetic ganglia.

Severe degeneration, atrophy, fibrosis, and necrosis of muscle have been described.[11, 12, 27, 30, 31] Although Smith, Ritchie, and Dawson [32] found only slight changes in experimental "trench foot," they stated that severe degeneration and regeneration occurred in "true frostbite." Blackwood [14] expressed the belief that Zenker's degeneration was present in an early case although he was concerned over the possibility that part of the change had occurred post-mortem. Siegmund [8] described waxy degeneration, hyalinization, myolysis, breaking up of the cytoplasm into droplets, anemic necrosis, and gangrene but found no resorptive cells in early cases. The cellular reaction, which was marked in one of the early cases reported here, may have been related to compression by a cast.

In the present series, encapsulated infarcts probably due to vascular occlusion and resembling those described in cases of Volkmann's contracture [33] were present as early as 32 days after exposure (case 5). The muscular degeneration which Blackwood and Russell [26] produced experimentally was of a different type. Although atrophy, which in case 6 was present 40 days after exposure, may have resulted from disuse, Blackwood attributed its occurrence to denervation. Siegmund described both patchy and diffuse atrophy, regeneration, and hyperplasia. Interstitial fibrosis has been considered to follow early edema [8] or rupture of degenerated fibers.[14]

Deformity and rigidity of the foot have been ascribed to damage of the short intrinsic muscles and their nerves with resultant unopposed tensions from the long muscles [4] and to sclerosis of tendon sheaths.[11] The secondary infection and inflammation of synovial surfaces which Rémy and Therèse [11] commented on were also observed in the present series. The frequent development of secondary infection with the usual pyogenic cocci and with other organisms, including those of tetanus and gas gangrene, is well known.[34, 35] The present group was not exceptional in this regard.

Bone

The only significant early changes in osseous tissues were reported by Siegmund,[8] who observed loss of nuclear staining and death of bone. He described the laying down of new bone about necrotic islands and dead lamellae and trabeculae in later cases, a process which was duplicated both in the late cases of the present series and in Ribbert's experimental material.[36] Circulatory disturbances have been held responsible for such osseous changes, which have been compared [8] to those of Sudeck's atrophy. Manteuffel [30] produced thickening of the shaft and widening of the marrow beneath a thinned-out epiphysis in his experiments. Although Brahdy [37] mentioned "dry necrosis," sequestration has seldom been observed.

In late cases osteoporosis may be so marked that the bones cut easily; [8] it can be demonstrated roentgenologically.[38, 39] The widening of the canals in the cortical bone, decrease and thinning of the trabeculae in the spongiosa, and broadening of the marrow spaces account for the rarefaction.[8, 11, 14] Blackwood stated that enough new bone may form to restore the normal roentgenographic appearance. Although productive periostitis and periosteal apposition of new bone have been noted, proliferation of bone is seldom active.[8] Rémy and Therèse described osteophytes at the edges of amputations. Strandell [40] stated that cartilage, and more specifically, the unclosed epiphysis, is especially sensitive to cold, but other workers have not mentioned this point.

In the marrow, edema, serous exudation, cellular infiltration, vacuolation, and myxomatous degeneration may occur.[8, 11, 14] Siegmund described residual bands of perivascular fibrosis in the poorly cellular connective tissue which replaced the gelatinous marrow. The marrow fat in the present series showed the same changes which characterized the adipose tissue in general.

Vascular Changes

The numerous physiologic studies of vascular responses to cold [41, 42] have shown that an initial vasoconstriction is followed by a marked reactive hyperemia. Davis and co-workers discovered by capillaroscopic studies [1] that the normal papillary loops were not demonstrable for as long as 24 hours after high altitude frostbite. Injury by cold is characterized also by an initial stage of spasm, which is succeeded by a hyperemic phase in which the extremities are hot and red,[43] the pulses are palpable,[44] and oscillometric pulsation may even be increased.[28, 45] The occasional conflicting reports of absence or diminution of the pulses [4, 46, 47] may have resulted from observations made either in the anemic vasoconstriction stage or in the later thrombotic phase. Norm-

ally, one reaction to cold is the opening of the arteriovenous anastomosis.[48, 49] Jochim and Hertzman [50] studied the vascular reaction to cold in the finger pad; they found that one group of subjects showed an excessive increase of blood volume before the amplitude of the pulse increased during the phase of reactive hyperemia. They expressed the opinion that arteriolar dilatation, in the absence of adequate opening of the anastomoses, favored the development of tissue damage through engorgement of the subpapillary venous plexus and consequent capillary stasis. Grant [51] was among the first to point out the rôle of the anastomosis in protecting the capillary bed. The histologic appearance of the vascular channels in the current material, especially in the peripheral and mummified portions, is consistent with the view of Jochim and Hertzman. On the other hand, Theis [52] stated that shunting of the blood through the anastomosis resulted in damage through failure of irrigation of the peripheral tissues. He attributed the changes in chronic frostbite to inflammatory involvement of the glomus structures, but his photograph is unconvincing. A satisfactory detailed anatomic study of the arteriovenous anastomoses in lesions produced by cold has not yet been reported. Jochim and Hertzman [53] expressed the belief that the absence of reactive dilatation and venous engorgement in the skin of the forearm resulted from the dearth of anastomoses in this region and suggested that the topography of the lesions in immersion foot might be related to the distribution of arteriovenous shunts.

The importance of transudation of fluid from congested vascular channels, in which permeability is presumably increased, has been stressed by many workers.[3, 54] Going on the theory that the nutrition of the tissues was interfered with by transudation, several [55–57] have suggested therapeutic incision. Smith, Ritchie, and Dawson [58] found that hemorrhage, in addition to exudation, took place especially after warming of the chilled tissues. Davis *et al.*[1] suggested that exudation accounts for the wet forms of thermal injury and that dry gangrene results from thrombosis, which prevents the development of transudation, but Rotnes and Kreyberg [59] attributed the plugging by residual cellular masses to loss of fluid from vessels.

There is no unanimity about the extent and significance of thrombotic occlusions. Thrombosis and organization as the result of different types of exposure and varying degrees of cold, both in human and in animal material, have been described by numerous workers.[7, 11, 16, 28, 30, 31, 60–65] Changes in viscera [66, 67] as well as in extremities have been recorded. Arterial and venous lesions ranging in extent from minor degrees of intimal thickening and endoangiitis to complete

obliteration have also been reported,[1, 16, 27, 28, 30, 39, 60, 61, 68, 69] although such changes have not always been interpreted as the consequences of thrombosis and organization.[15] Leriche and Kunlin [28] stated that they had demonstrated vascular occlusion by roentgenologic methods, but Pässler [45] reported observations to the contrary.

A number of workers [5, 14, 32, 70, 71] have denied the occurrence of thrombi and vascular occlusion after cold injury or have belittled their importance in the genesis of tissue damage in clinical and in experimental material. Blackwood [14] stated that significant vascular changes in the cases of immersion foot which he studied were present only in relation to areas of gangrene and chronic infection and that the minor changes in large arteries were compatible with the age and previous physical activity of the patients. Analysis of the material which he studied may provide a partial explanation of his failure to find lesions comparable to those described in Siegmund's report [8] and in the present paper. The few entire extremities which he received for study were not gangrenous, and in most instances he was limited to examination of gangrenous toes, small portions of tissue, or stumps.

Those who have agreed that thrombosis does occur have differed among themselves as to the particular type of injury by cold in which it appears. Some have insisted [71, 72] that thrombosis takes place only after exposure to "wet cold"; others have stated [73, 74] that it occurs only as a result of injury by "dry cold"; and Talbott [75] claimed that freezing of an extremity was necessary to produce thrombosis. Blackwood,[14] a proponent of the view that thrombi and involvement of vessels are of little significance in the pathogenesis of lesions due to cold, agreed that obliterative endarteritis was a feature of late frostbite.

Greene's experiments [72] with dry cold confirmed a previous observation [50] that clumping of red cells and obstruction of dilated vessels were features of the initial reaction to cold injury. He stated that these clumps were not true thrombi and suggested that the "thrombi" described by earlier workers may have consisted of such cellular masses. Since many of the earlier investigators who described vascular plugs did not include photographs in their reports, evaluation of their observations is difficult, but it is perfectly proper to refer to agglutinations of red cells as thrombi [76] despite the scarcity of fibrin. The amorphous masses of red cells and platelets which were abundant in the early cases of this series resembled both those described by Siegmund [8] and those in the experimental material of Rotnes and Kreyberg [59] and Greene.

The organization, recanalization, and development of obliterative vascular lesions reported in Siegmund's study [34] of thermal lesions among the German troops on the Russian front were duplicated in the

late cases of the present group, although the injuries on the Eastern front were doubtless incurred during freezing weather. He stressed the purely endoangiitic nature of the involvement, even of large arteries well above the zone of demarcation, but admitted that some vascular changes, particularly in areas of gangrene and infection, could have resulted from extension of the inflammatory process. He expressed the belief that mural and intimal thickening might develop after a long period of vasoconstriction. Rémy and Therèse [11] and Siegmund described a train of events leading from hemorrhage and serous exudation to cellular proliferation, formation of mucinous ground substance, elastica, and collagen, and accumulation of phagocytes containing fat and hemosiderin in the vessels.

It seems probable that the vascularized polypoid intravenous vegetations and networks containing hemosiderin, which were stressed by Rémy and Therèse,[11] described in the present paper, and illustrated in other reports,[14, 39] developed from valvular hemorrhages and thrombi. Edwards and Edwards [77] described comparable venous changes in cases of Buerger's disease.

Local hemolysis with staining of tissues and vascular contents has been repeatedly described.[11, 39, 64, 72, 74] Systemic hemolysis, hemoglobinemia, and increased fragility of red cells may also occur under certain conditions.[13, 31, 78] In the experiments of Smith, Ritchie and Dawson, phagocytosis of red cells and leukocytes in regional lymph nodes was noted.[32]

Pathogenesis

It is not clear why necrosis begins despite a presumably adequate if not excessive blood supply. Possibly the tissue damage is a consequence of the poorly controlled blood flow, which, unregulated by the usual vasomotor machinery, is neither paced to the needs of the tissues nor properly routed. The importance of the arteriovenous shunts in this regard has already been discussed. Furthermore, since blood flow in the deep portions of an extremity does not always parallel that in the tissues near the surface,[79] the superficial hyperemia may not accurately reflect the state of the circulation in the muscle. The cramps described by Patterson [4] may result from ischemia involving the nerves as well as the muscles in the deep portions of the limb.

The small arteriovenous differences in oxygen content observed at low temperatures [80, 81] are due to shunting through arteriovenous anastomoses and to diminution in the amount of oxygen released to the tissues. Since chilled tissues require little oxygen, it seems unlikely that simple anoxia is responsible for early tissue damage. Lewis and Love [82] and Lake [9, 54] found that obstructing the circulation to tissues damaged

by cold resulted in less severe injury. Ischemia may act by inhibiting the harmful excessive hyperemia and transudation associated with the reactive vascular response to cold. Fell and Hanselman [83] reported that pressure dressings prevented shock and death after experimental freezing. In the dissenting report of Levin and Khalezkaya [84] it is stated that arterial obstruction and ischemia made experimental frostbite worse. Nevertheless, cooling of the damaged extremity, which reduces both metabolism and blood flow, is an accepted form of therapy.[85] The injurious effect of warming, early sympathectomy, or any measure resulting in excessive vasodilation, is generally known; [86] the idea has been advanced that increased warmth also speeds up the production of harmful metabolites.

The topography of the lesions in trench foot sometimes bears a striking relation to the regions in which there was pressure by footwear.[87] Safford and Nathanson [88] have commented on the development of local necrosis in chilled tissues at points of pressure, but the manner in which compression participates in the production of the lesions is not clear. Lack of active movement, such as the enforced immobility encountered in trench warfare, has been said to result in incomplete emptying of the veins and lymphatics in the already chilled tissues.[89]

It is not known whether the early clumping of red cells represents true agglutination or is merely the result of loss of fluid from the blood. Cold hemagglutination has been described in cases of gangrene of the extremity [90] and Raynaud's disease.[91] Greene [72] found hemolysis a frequent, if not constant, feature in his experiments with frostbite, and the occurrence of local and systemic hemolysis in cold injury has already been mentioned. Consideration should be given to the possibility that released hemoglobin may affect blood vessels directly and that masses of residual stroma from destroyed erythrocytes may block the circulation.

Wieting's report,[61] which includes one of the first pathologic descriptions of trench foot, was entitled "Gefässparalytische Kältegangrän," and the older literature is replete with references to the occurrence of "cold neuritis" in association with frostbite and trench foot. The anesthesia which occurs early as a result of neuritis may lead to "trophic" lesions. The recently developed but cumbersome term "vasoneuropathy" suggests the importance of neural elements in the vasomotor paralysis and the damage to the reflex vasoconstrictor mechanism which follow undue exposure to cold.[3, 9, 92] Study of the present group of cases has failed to reveal involvement of the sympathetic fibers in the main nerve trunks. The medullated fibers, which are said to carry antidromic vasodilator impulses but not vasoconstrictor

stimuli,[93] showed profound alterations, even in the early cases. Blackwood [94] also noted sparing of the small myelinated and unmyelinated fibers. Disturbances of sweating and the sensitivity to adrenalin in cases of immersion foot [43, 95] have been taken as evidence of injury to the sympathetic nerves. Siegmund [8] stated that he was "inclined to believe" in the occurrence of direct damage to the "vegetative terminal reticulum" and the adventitial nerve branches, although he presented no unequivocal anatomic evidence. It is obvious that detailed studies of the sympathetic nerves in injury by cold should be undertaken.

In view of the diffuse damage to all structures in cases of injury by cold, the possibility that low temperature may exercise a direct influence on tissue cannot be overlooked. Despite the existence of temperature gradients [96, 97] between the skin surface and the tissue strata and the insulating action of the fat layer, the deep tissues may become surprisingly cold.[98, 99] Haxthausen [73] stated that cold affects the subcutaneous structures of man more severely than those of experimental animals. Although the mural angiitis in the early cases in this series probably resulted from vascular obstruction, the possibility of a direct effect of cold cannot be ignored. It is known that under certain conditions vessels react directly to low temperature. Angiitis occurs [19, 100, 101] in pernio and chilblains, and a necrotizing arteriolar reaction in perniosis has been observed.[24] However, Siegmund [8] pointed out that the spottiness of the vascular changes spoke against the possibility of a direct effect of cold on vessels. Safford and Nathanson [88] suggested that the damage caused by cold is in reality a "burn" resulting from the sharp rise in temperature which occurs after exposure. Similarities in the lesions caused by burning and by cold obviously exist.[57]

It has been suggested that some persons have a predisposition to injury by cold. It is interesting to speculate about the fact that only a few of the many subjects exposed suffer damage. Some studies [46,102, 103] have apparently indicated the presence of predisposing constitutional elements, but others [104] have disclosed no evidence of such factors. The high incidence of epidermophytosis among soldiers suffering from gangrene after cold [4] brings to mind the now abandoned theory, voiced during the last war by some French authors, that fungus infection was responsible for trench foot.[105] The suggestion that persons who had previously lived in warm climates were more likely to suffer from immersion foot has likewise been abandoned. Jochim and Hertzman [50] noted that some experimental subjects exhibited a vascular response to cold which could be considered abnormal, an observation which doubtless will open up new lines of investigation.

In the early cases of the present series there was no evidence of

antecedent chronic vascular disease, such as arteriosclerosis or endarteritis. Pathologists occasionally encounter cases of gangrene, apparently due to cold, in which it is impossible to decide whether the patient had peripheral vascular disease, such as thromboangiitis obliterans, which was aggravated by frostbite, or whether previous exposure to cold had resulted in vascular sclerosis. Siegmund [8] stated that the late vascular changes in frostbite did not differ from other forms of endangiitis obliterans. The possible relation between injury by cold and Buerger's disease has been mentioned repeatedly.[15, 28, 29, 68, 69, 106, 107] However, other workers,[52, 108] including Buerger,[109] have insisted that many cases of peripheral vascular disease with gangrene had been erroneously considered instances of cold injury. Nevertheless, it has been established that obliterative vascular lesions may follow damage by cold; they may play a rôle in the production of the sensitivity to cold and the disturbance in the neurovascular mechanism which are residua of cold injury. The profound changes in the subcutaneous panniculus described in the present series may also participate in the genesis of the sensitivity to cold.

It has been conventional to differentiate between "true frostbite" and injury produced by chilling under conditions in which the freezing point was not reached. Injury from direct freezing has been attributed to the release of thrombokinase from thawing red cells and subsequent thrombosis [54] and to the release of H-substance from tissue damaged by ice crystals.[110] Rupture of cell membranes, high concentrations of unfrozen salts, desiccation, and disturbances of gel-sol relations have been suggested as means by which tissue is injured.[88] Kochs [111] observed diffusion currents about melting crystals in thawing tissues. Exposure under the weather conditions obtaining after a thaw (Frostschäden ohne Frostwetter) [112] has usually resulted in more injuries than are incurred in actual freezing weather.[113] "Wet cold" is especially damaging because water has a high capacity for absorbing heat (27 times that of air).[98] Injury under such conditions has usually been considered to result from the vascular occlusion which follows chilling rather than from cold directly. Even in "true frostbite," "solidification" may be due to freezing of intercellular fluids without cellular injury,[9] and supercooling [110] may enable tissues to stand extremely low temperatures without freezing. Lake [9] stated that there is often only a small focus of "true frostbite," that the deeper tissues rarely freeze, and that the major part of the tissue reaction, even in cases of true frostbite, may be of the secondary neurovascular type which follows chilling. Other workers [74, 114] have pointed out that frozen tissues are not brittle, and Greene [70] recently voiced the hope that "the theory of solidification

may finally receive decent burial" after having shown experimentally that solidified tissues do not necessarily die. The recent attempt by Davis and co-workers [1] to differentiate between high altitude frostbite and ordinary frostbite was criticized by Safford and Nathanson [88] and by Greene.[70] Until more is learned about the reactions of tissue to low temperature, it may simplify matters to put all injuries caused by cold in one category, since they have comparable clinical and pathologic features.

Although the present study confirms the view that the generalized tissue changes which take place after injury by cold result from the vascular lesions which Siegmund [8] termed "thromboangiopathy" and which Staemmler [115] appropriately described as "anatomic fixation" of the functional disturbances induced by low temperature, a number of points demand study and clarification. There is need for further investigation of the earliest histologic changes which occur immediately after exposure to cold, especially those involving the arteriovenous anastomoses and the sympathetic nerves supplying blood vessels, and closer study of the reactions in adipose tissue. These are only some of the problems which are susceptible to attack by the pathologist; the problems which await the attention of the physiologist and clinician are legion.

Summary

The morphologic changes in 14 recent cases of trench foot have been presented. The conclusion has been reached that all injuries resulting from exposure to low temperatures exhibit a common pattern and result from a similar train of events.

The essential early change is a disturbance in the circulatory mechanism; the consequent stagnation of blood leads to thrombosis and, subsequently, to gangrene, which in many ways resembles ordinary peripheral ischemic necrosis complicated by secondary infection but has certain unusual features. Particular attention has been called to the occurrence of agglutinative thrombosis, profound changes in the fat, and interesting neuromuscular and osseous alterations. The delayed sensitivity to cold which follows apparent recovery may be caused in part by the damage to the subcutaneous panniculus and is certainly related to the occlusive peripheral vascular disease.

Further morphologic studies can contribute to an understanding of the pathogenesis of trench foot. Investigation of the early changes in the myelin sheaths and the fat of the subcutaneous panniculus will determine whether tissues rich in lipoid are specially sensitive to cold. Detailed examination of the sympathetic fibers which supply blood vessels and of the arteriovenous anastomoses will decide whether the initial lesion is vascular or neural.

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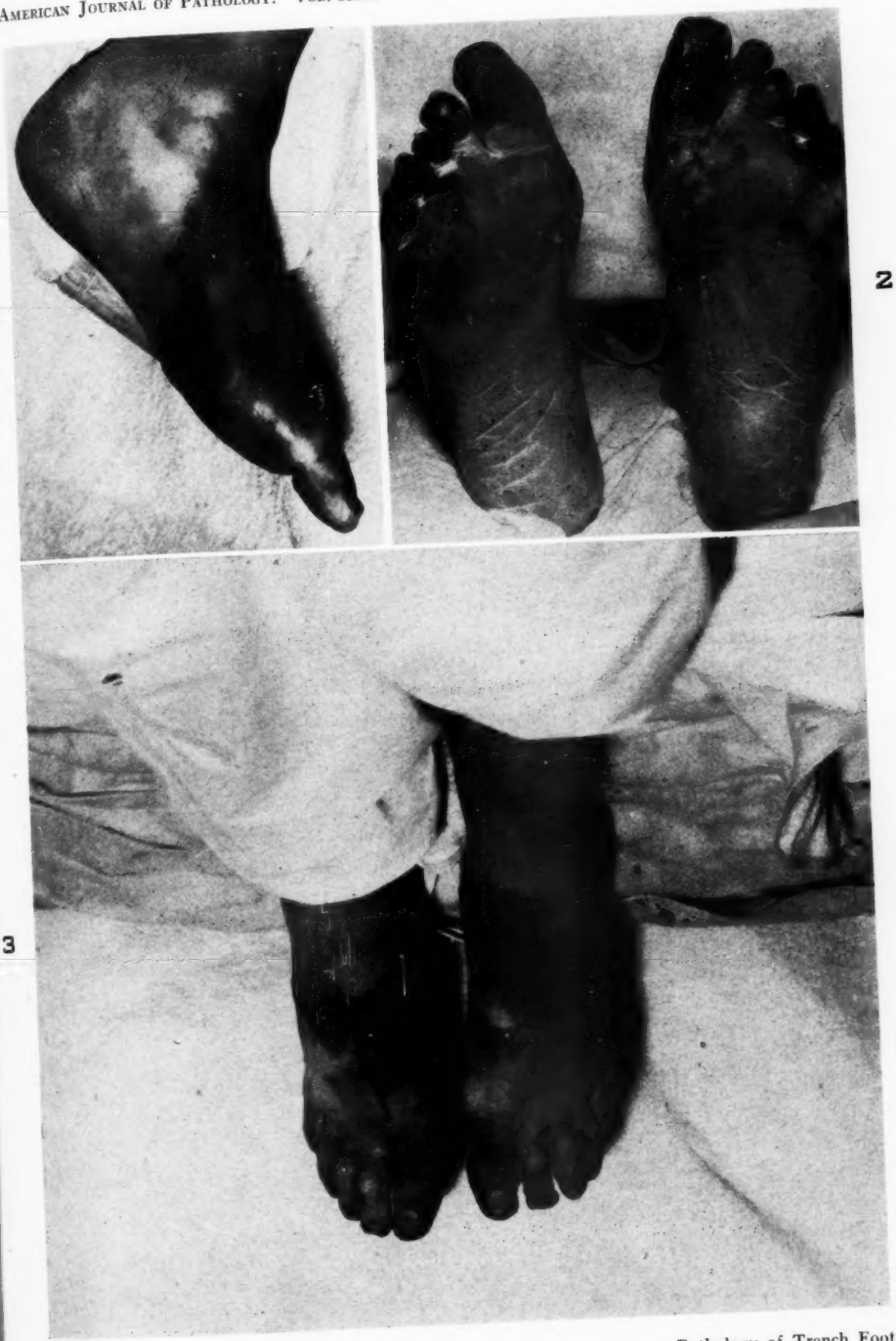
DESCRIPTION OF PLATES

PLATE 68

FIG. 1. (A.M.M. Neg. No. 82235. Case 3). Hemorrhage, cyanosis, and edema of the foot, 5 days after exposure. The sharp line separating the involved and normal tissues is evident. This appearance is typical of the early changes observed in the present series.

FIG. 2. (A.M.M. Neg. No. 82240. Case 7). Dry gangrene of the toes in a case of severe trench foot, 17 days after exposure. The toes later sloughed.

FIG. 3. (A.M.M. Neg. No. 82238. Case 5). Necrosis of the skin, 12 days after exposure. The blackened, injured tissues are sharply demarcated from the uninjured portion of the limb at the malleoli, the level at which the zone of involvement terminated in most cases.



Pathology of Trench Foot

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PLATE 69

FIG. 4. (A.M.M. Neg. No. 82050. Case 1). Thick section of skin from an early lesion. The papillary vessels are dilated, tortuous, and engorged. Hematoxylin and eosin stain. × 110.

FIG. 5. (A.M.M. Neg. No. 82229. Case 10). Section of skin from a mummified region in a late case. Shrinkage and distortion of the epidermis and subcutaneous collagen are marked. Vascular dilatation and engorgement have persisted; hyalinized and hemolyzed masses of red cells plug some vessels. Hematoxylin and eosin stain. × 110.

FIG. 6. (A.M.M. Neg. No. 81952. Case 9). Degeneration of the subepidermal connective tissue in a late case. The elastica is frayed and fragmented. Weigert's elastica and van Gieson's stains. × 190.

FIG. 7. (A.M.M. Neg. No. 78629. Case 9). Phagocytosis of fat in the subcutaneous adipose tissue in a late case. Foam cells are present throughout the lobule. Hematoxylin and eosin stain. × 145.

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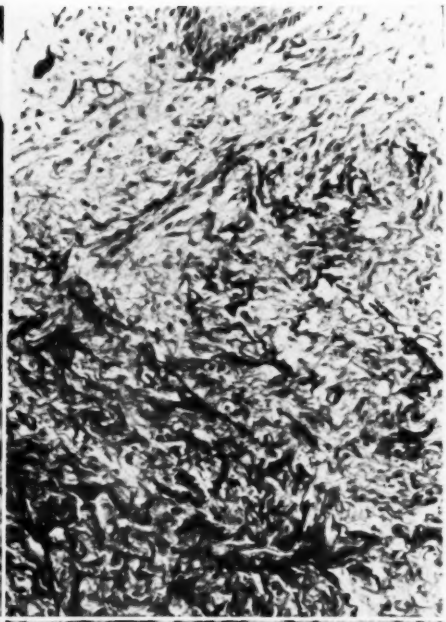

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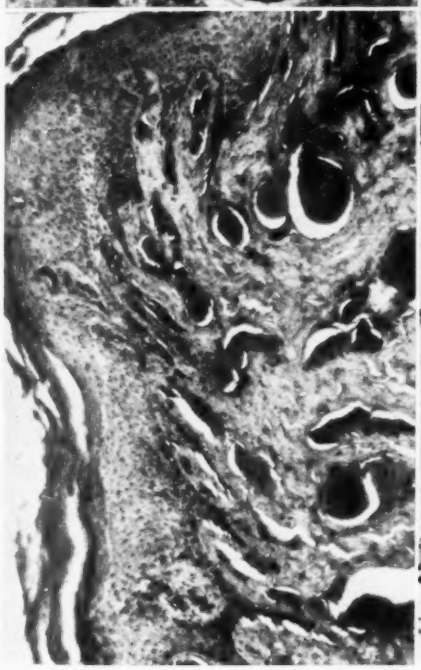

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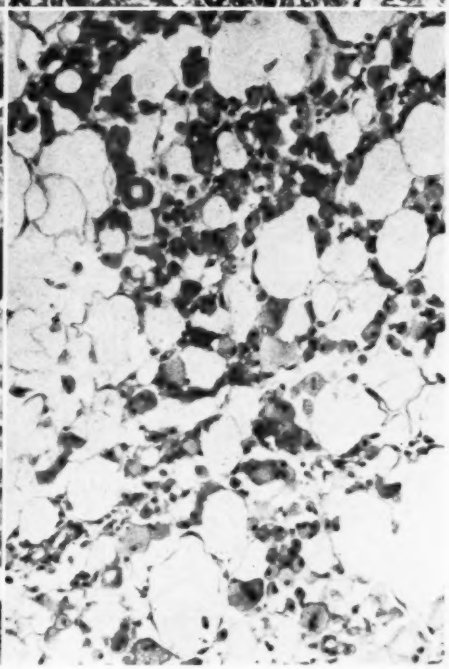

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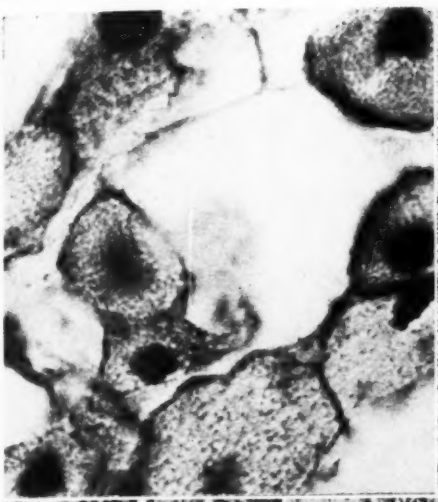
PLATE 70

FIG. 8. (A.M.M. Neg. No. 81141. Case 9). High-power view of the lipoid phagocytes shown in Figure 7. Masson's trichrome stain. × 1360.

FIG. 9. (A.M.M. Neg. No. 78360. Case 8). An oil cyst, lined by foam cells, in the subcutaneous fat in a late case. Hematoxylin and eosin stain. × 205.

FIG. 10. (A.M.M. Neg. No. 81954. Case 11). Connective tissue elements are replacing the cells of a subcutaneous fat lobule in a late case. Hematoxylin and eosin stain. × 145.

FIG. 11. (A.M.M. Neg. No. 81054. Case 8). Atrophy and inflammation of subcutaneous fat lobules in a late case. The interlobular fibrous septa are thickened. Hematoxylin and eosin stain. × 30.



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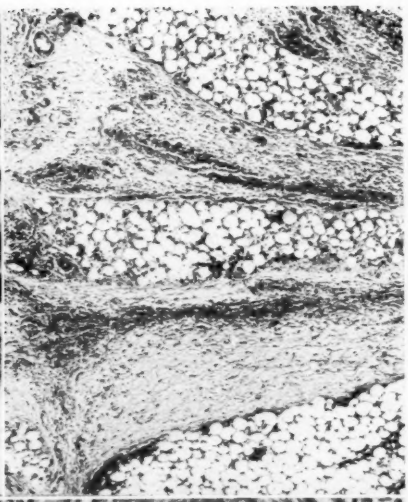

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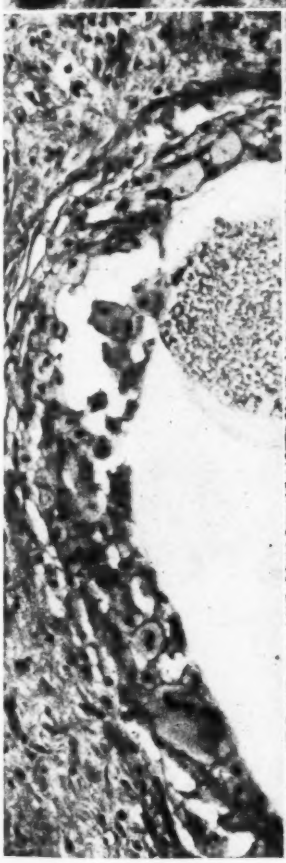

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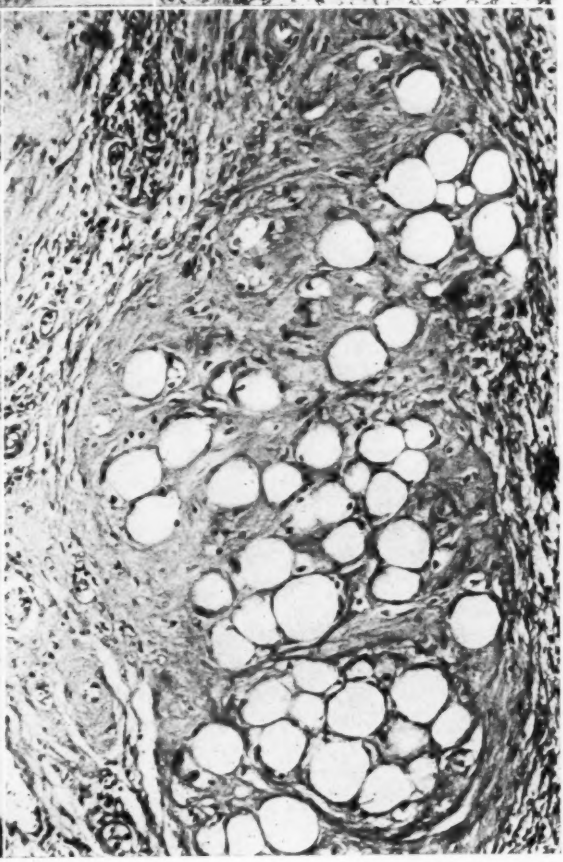

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Pathology of Trench Foot

Plate 71

Fig. 12. (A.M.M. Neg. No. 82059. Case 3). Thrombosis of posterior tibial vein in an early case. The thrombus consists almost entirely of agglutinated red cells. Hematoxylin and eosin stain. × 65.

Fig. 13. (A.M.M. Neg. No. 82054. Case 1). Obstruction of the lumen of a small vessel in the subpapillary plexus by a granular mass of platelets. The section was taken from an early lesion. Hematoxylin and eosin stain. × 500.

Fig. 14. (A.M.M. Neg. No. 82187. Case 1). Thrombosis of the posterior tibial artery in an early case. The thrombus is composed of agglutinated masses of red cells and a framework of platelets. Weigert's elastica and van Gieson's stains. × 32.

Fig. 15. (A.M.M. Neg. No. 81964. Case 5). Thrombosis of an artery from the gangrenous foot in a late case. Organization of the thrombus and hemorrhage into the vessel wall are shown. Hematoxylin and eosin stain. × 50.

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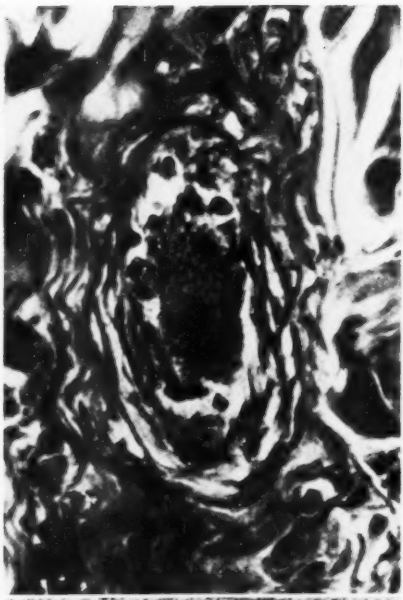

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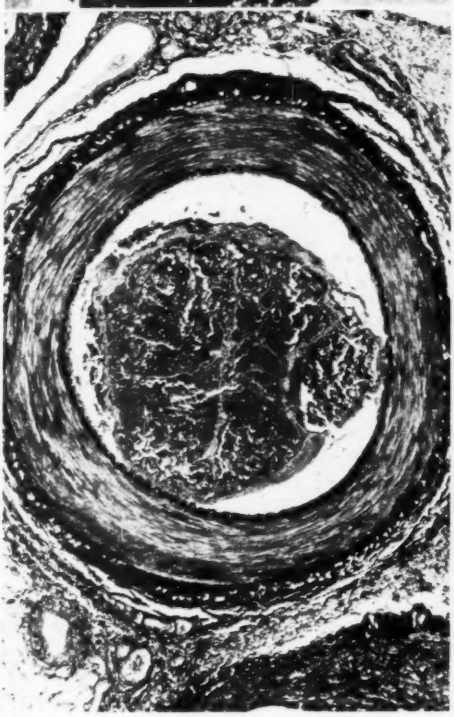

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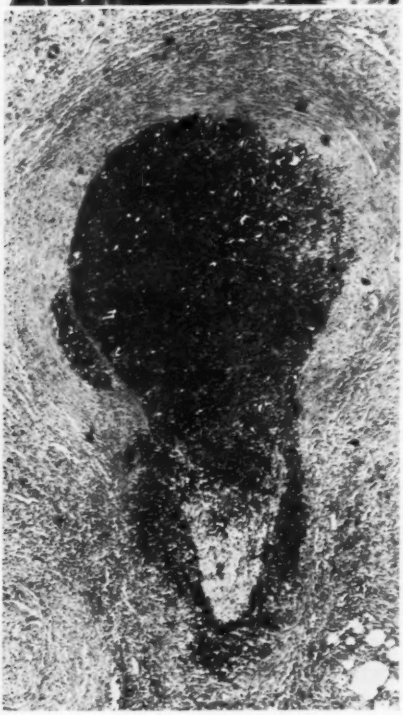

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Pathology of Trench Foot

PLATE 72

FIG. 16. (A.M.M. Neg. No. 82186. Case 1). Mural angiitis in an early case. The wall contains chromatin débris and is infiltrated by leukocytes. The muscular elements are degenerated and the media contains eosinophilic granular material. Hematoxylin and eosin stain. × 500.

FIG. 17. (A.M.M. Neg. No. 82518. Case 3). Constriction of posterior tibial artery in an early case. Weigert's elastica and van Gieson's stains. × 32.

FIG. 18. (A.M.M. Neg. No. 81950. Case 5). Longitudinal section of a plantar artery in the 32-day case. Organization is under way at the head of a thrombus. Hematoxylin and eosin stain. × 75.

FIG. 19. (A.M.M. Neg. No. 78625. Case 8). Proliferation of intima and mucinous degeneration in a small artery. Hematoxylin and eosin stain. × 175.

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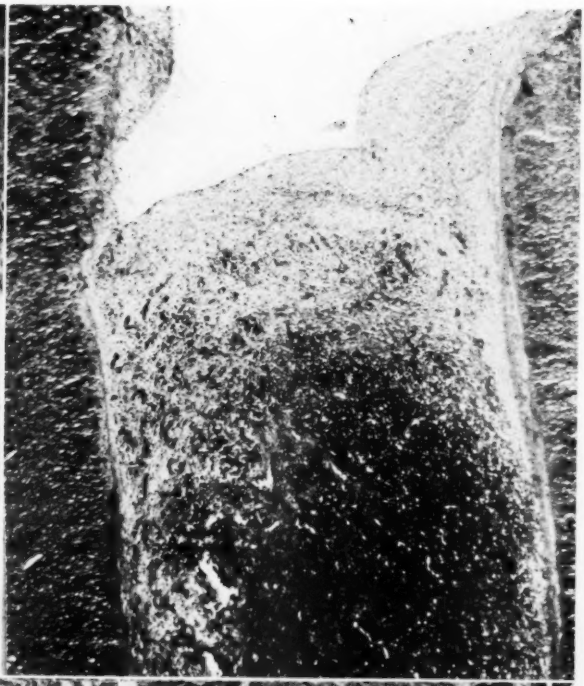

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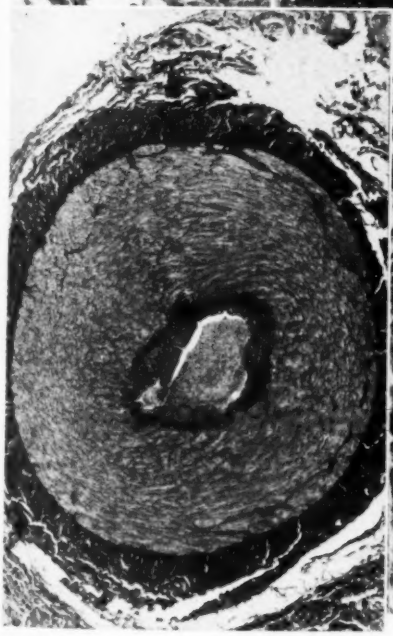

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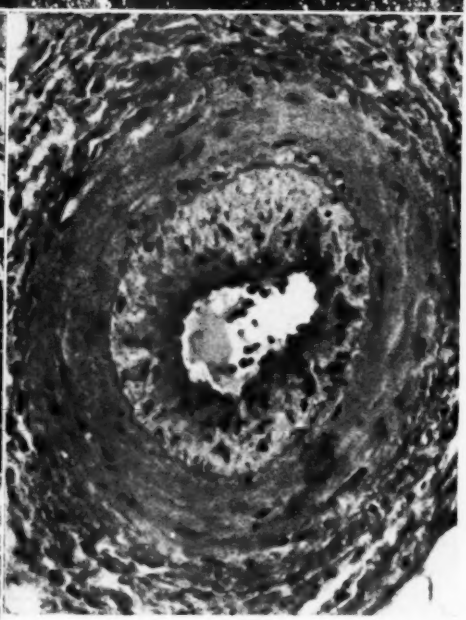

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PLATE 73

FIG. 20. (A.M.M. Neg. No. 78631. Case 8). Obliteration of the lumen of an artery in a late case. The inner elastic membrane is ruptured; the media and adventitia are scarred. Hematoxylin and eosin stain. × 175.

FIG. 21. (A.M.M. Neg. No. 82190. Case 10). Recanalization of a small obliterated artery. Many new channels, some with muscular walls, have formed. Hematoxylin and eosin stain. × 280.

FIG. 22. (A.M.M. Neg. No. 81139. Case 9). Organization and recanalization of an artery in a late case. The elastica is ruptured, frayed and distorted. Weigert's elastica and van Gieson's stains. × 145.

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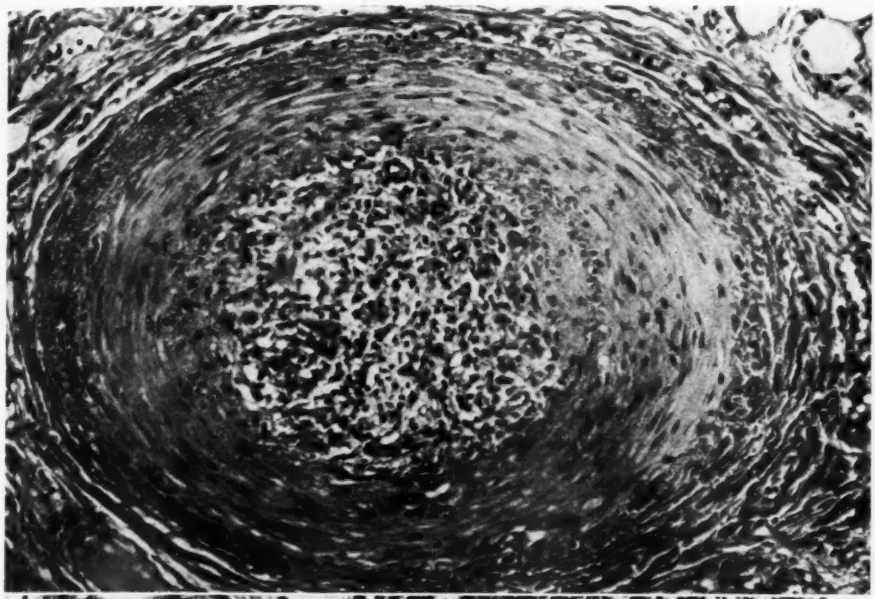

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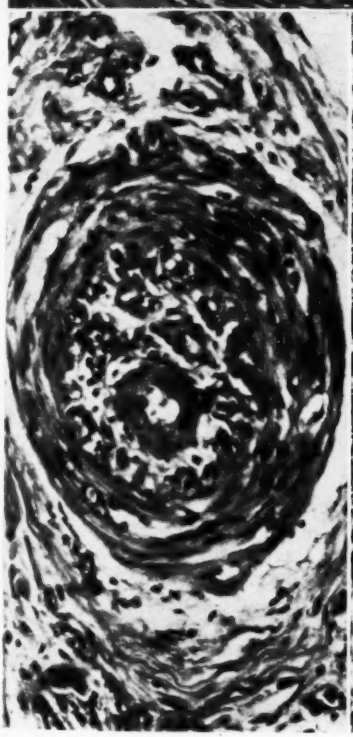

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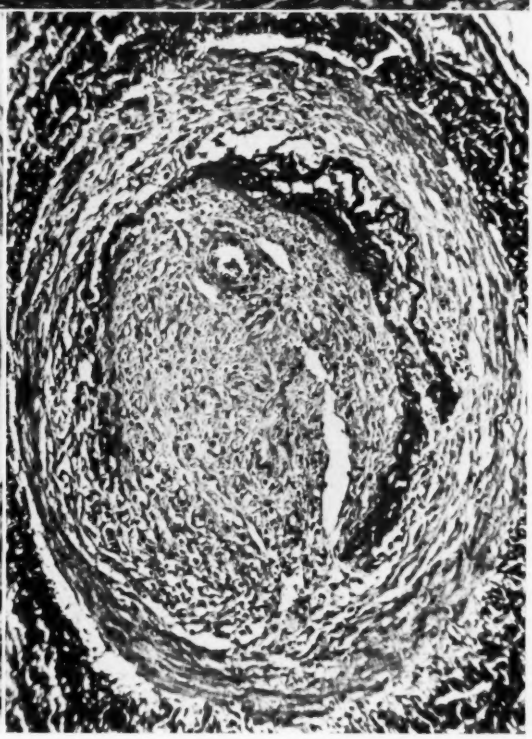

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Pathology of Trench Foot

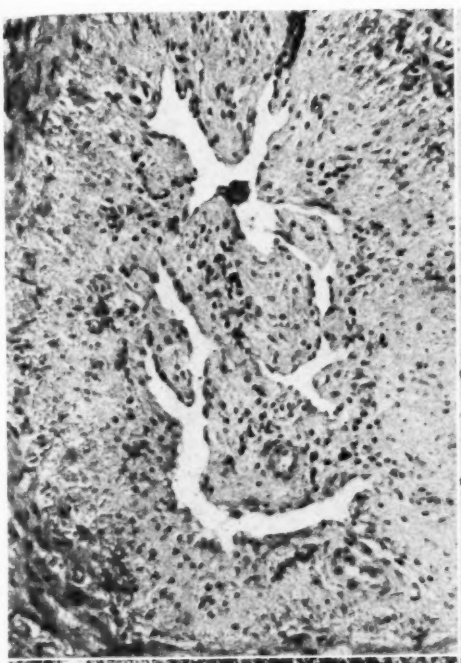
Plate 74

Fig. 23. (A.M.M. Neg. No. 81958. Case 10). Phlebosclerosis, intimal proliferation, and labyrinthine recanalization in a late case. Hematoxylin and eosin stain. × 90.

Fig. 24. (A.M.M. Neg. No. 81968. Case 10). Polypoid endophlebitis. Newformed channels with muscular walls traverse the proliferated strands. The dark masses are phagocytes laden with hemosiderin. Hematoxylin and eosin stain. × 145.

Fig. 25. (A.M.M. Neg. No. 81948. Case 5). Necrotic focus in muscle, encapsulated by fibrous tissue and inflammatory elements. Muscle infarcts of this type resemble those seen in instances of Volkmann's contracture. Hematoxylin and eosin stain. × 75.

Fig. 26. (A.M.M. Neg. No. 78633. Case 9). Atrophy of muscle in a late case. The shrunken fibers are widely separated. Hematoxylin and eosin stain. × 145.



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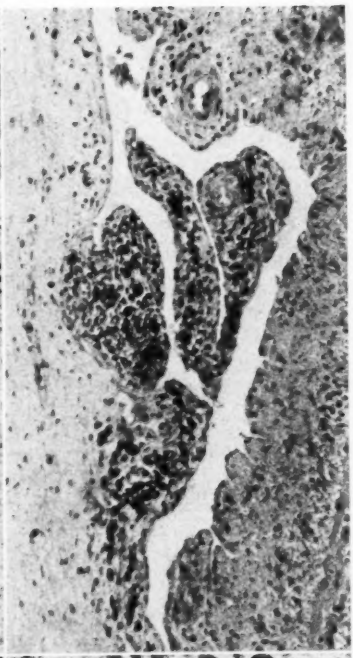

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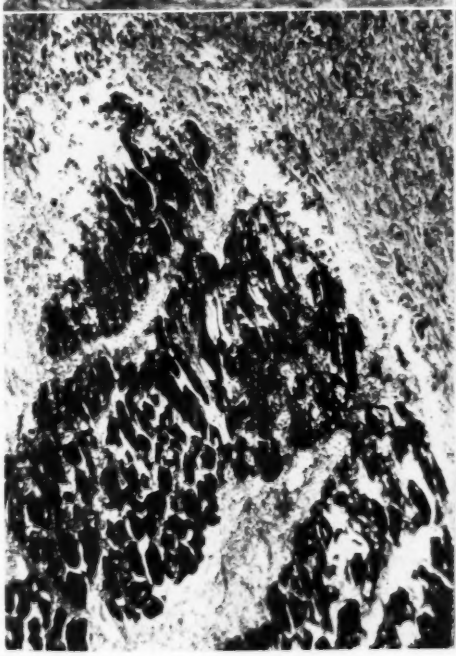

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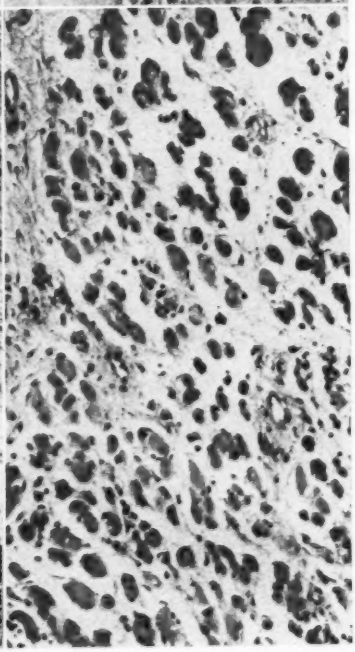

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PLATE 75

FIG. 27. (A.M.M. Neg. No. 82864. Case 1). Degeneration of posterior tibial nerve in an early case. The axis cylinders of the large medullated fibers are broken into tortuous segments. Bielschowsky's stain. × 910.

FIG. 28. (A.M.M. Neg. No. 82862. Case 1). Degeneration of the dorsal pedal nerve in an early case. The axis cylinders are fragmented and beaded. Bielschowsky's stain. × 910.

FIG. 29. (A.M.M. Neg. No. 82523. Case 1). Demyelinization of the medial plantar nerve in an early case. A few segments of myelin are all that remain along the fibers. Frozen section. Spielmeyer's stain. × 500.

FIG. 30. (A.M.M. Neg. No. 82531. Case 1). Cross section of portion of the posterior tibial nerve in an early case. The groups of small fibers are undamaged. Bielschowsky's stain. × 500.

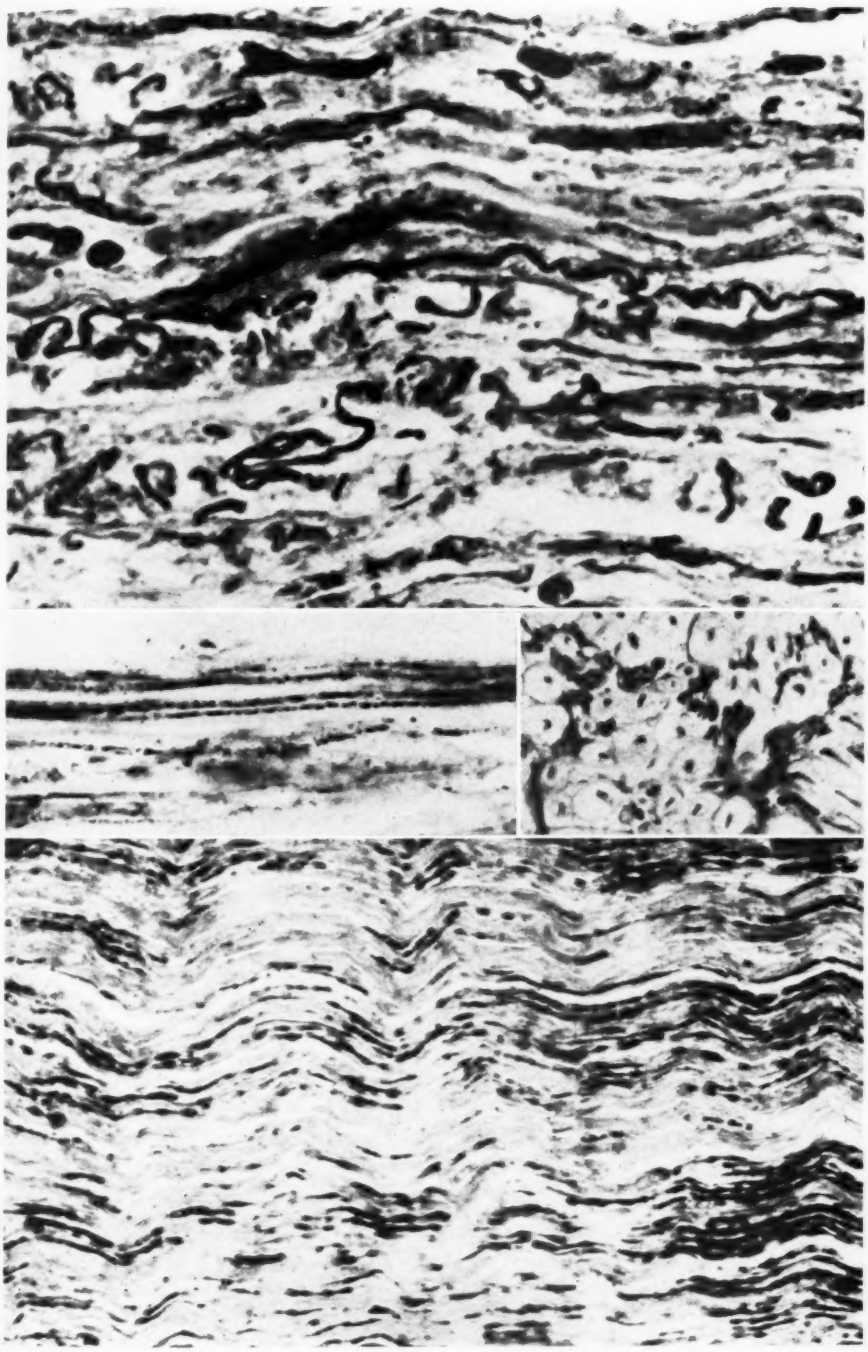

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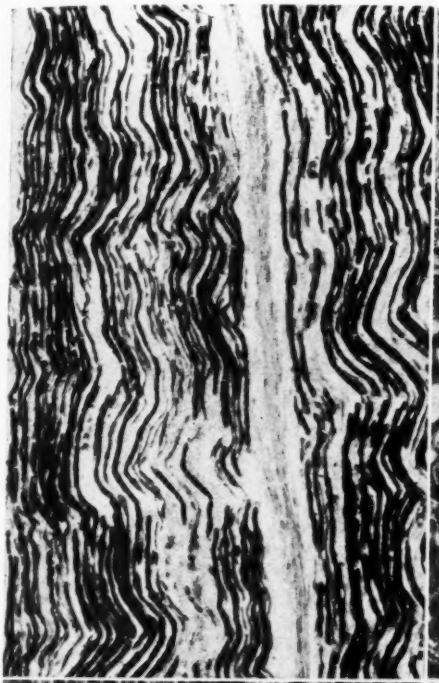
Fig. 31. (A.M.M. Neg. No. 77251. Case 8). Slight demyelinization of nerve above the region of gangrene in a late case. Frozen section. Spielmeyer's stain. × 125.

Fig. 32. (A.M.M. Neg. No. 77253. Case 8). Marked segmentation, beading, and loss of myelin in a nerve from a region of gangrene. This section is of the same nerve as is illustrated in Figure 31, but it was taken at a different level. Frozen section. Spielmeyer's stain. × 220.

Fig. 33. (A.M.M. Neg. No. 81956. Case 13). Lipoid phagocytosis in a degenerated nerve in a late case. Foam cells are scattered between the damaged fibers. Weigert's stain. × 230.

Fig. 34. (A.M.M. Neg. No. 82524. Case 8). Degeneration of nerve in a late case. Many axis cylinders have been lost and those which remain are ballooned. Bielschowsky's stain. × 550.



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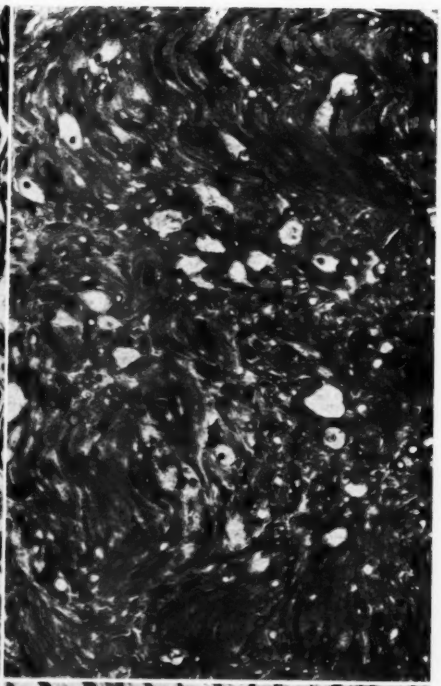

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PLATE 77

FIG. 35. (A.M.M. Neg. No. 81957. Case 11). Sclerosis of nerves in a late case. The nerve bundles are embedded in dense fibrous tissue. Hematoxylin and eosin stain. × 175.

FIG. 36. (A.M.M. Neg. No. 81960. Case 9). Necrosis of bone. Dead trabeculae with empty lacunae have been sheathed by viable bone. Osteoblasts line the newly formed trabeculae. The marrow is fibrotic. Hematoxylin and eosin stain. × 145.

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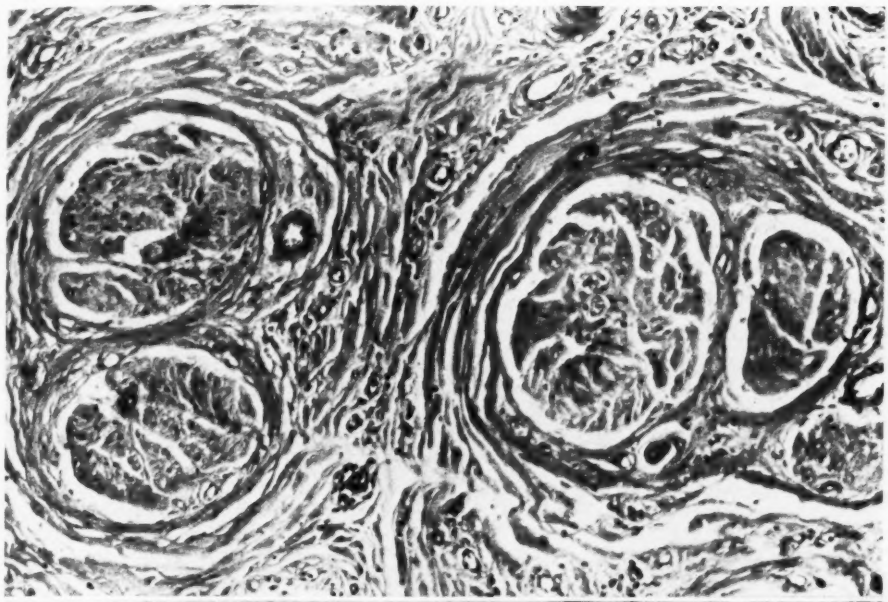

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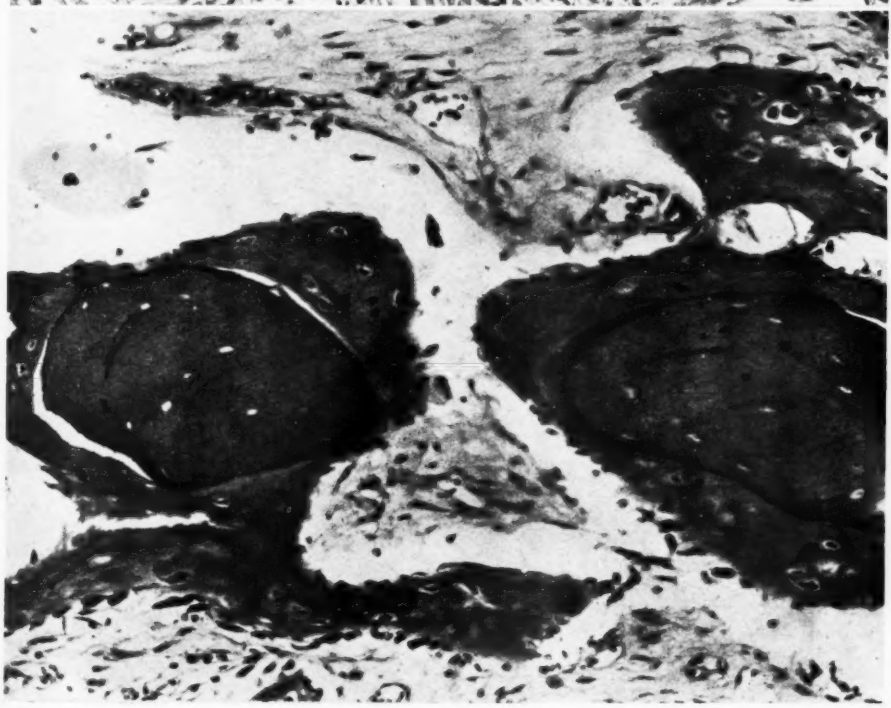

Friedman Pathology of Trench Foot

INTRANUCLEAR INCLUSIONS IN PANLEUKOPENIA OF CATS

A Correlation with the Pathogenesis of the Disease and Comparison with Inclusions of Herpes, B-Virus, Yellow Fever, and Burns *

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The early publications of Lawrence, Syverton, Shaw, and Smith [1] and of Hammon and Enders [2, 3] stimulated us to investigate in more detail the intranuclear inclusions in the mesenteric lymph nodes and in the intestinal epithelium of animals with the disease which they described. This disease has been named spontaneous or infectious feline agranulocytosis,[1, 4] malignant panleukopenia of cats,[3] infectious aleukocytosis of cats,[5] and perhaps we should include also the older clinical designation of feline enteritis.[6, 7] Most of the previous studies made have been devoted to the blood picture. The gross and microscopic appearances have been studied in nearly every case at the height of the disease or some time thereafter and not during developmental stages. The only exceptions are some examinations of bone marrow [1] and lymph nodes.[3] It is our opinion, therefore, that the data to be reported here help to decide which tissues show primary and which secondary reactions, whether red blood cells are involved in either reaction, and also assist in correlating the degree of illness exhibited by the cat with the changes going on within its body, and the extent of gross and histologic lesions with cellular alterations at different stages of the disease. This material permits also a description of the various types of intranuclear inclusions associated with the malady, and of the cycle of ageing which each type follows. When this was done it was possible to compare and classify these inclusions with those produced by other viruses and to evaluate the usefulness of each type for the diagnosis of panleukopenia of cats.

Materials and Methods

The cats used in this work constituted three groups: 2 well cats, 12 post mortem or *in extremis* from the clinic, and 23 receiving the virus under experimental conditions. The well cats were classified as such for comparative purposes in that they did not show symptoms of panleukopenia. Some had intestinal parasites. Animals in the three

* Received for publication, June 19, 1944.
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groups represented various breeds and hybrids and usually little or no data about age or previous history of disease were available.

Passage of virus in the preliminary experiments was accomplished by contact of the well animal with one having the disease; keeping them in the same room, but separated, was sometimes sufficient. The virus was passed also by intraperitoneal inoculation of blood or spleen, or spleen and kidney.

Many experiments were failures for one reason or another but the last experimental group gave more usable data than any other and the history of the animals (cats 79 to 83) and conditions of that experiment are presented since these cats will be referred to frequently in the observations which follow. Cats 79, 80 and 81 were litter-mates, said to have been born on April 1, 1943. They were received at the laboratory on October 8, 1943. All were females, all tabbies, and weighed 2425, 2210 and 2445 gm. respectively. Cat 82 was a stray which walked into the laboratory the day the others were received. Its history was not known but it was also a tabby, female, and weighed 1850 gm. The source of the virus used was a spleen received on October 14, 1943, from the Pitman-Moore Co.* by air express, frozen in dry ice. Their cat no. 3835, with a diagnosis of panleukopenia, had been killed on October 6, 1943. Inoculation was made the day the virus was received. The spleen, ground with sterile sand and Locke's solution to make a 20 per cent suspension, was centrifuged for 30 seconds at about 2000 r.p.m. From the supernatant fluid 0.5 cc. was injected intraperitoneally. After each injection a drop or two was cultured on nutrient agar. All cultures remained negative for 4 days, but the culture from cat 80 on the fifth day showed two small colonies. One was identified as *Staphylococcus albus* and the second could not be identified by the tests run, but was not considered to be a common pathogen.†

It was planned to remove tissues from cats killed at intervals of 3 days. At each period the animal was chosen which, on the basis of leukocyte count and general reactions, seemed the sickest.

Most tissues were fixed in Zenker's fluid with 5 per cent acetic acid. For some of the earlier clinical cases 10 per cent formalin was used. The stains employed were hematoxylin and eosin, Giemsa's, phloxine and methylene blue, Masson's trichrome stain, and Feulgen's thymonucleic acid reaction, without or with a counterstain of fast green or orange G. Giemsa's stain was variable in its results which seemed to be influenced by the length of time the tissues had remained in Zenker's

* We are indebted to Dr. W. S. Gochenaur of the Pitman-Moore Co., Indianapolis, Ind., for preparing this material for our use and shipping it to us.

† We are indebted to Dr. R. A. Packer of the Department of Veterinary Hygiene, Iowa State College, who kindly ran the identification tests and gave his findings.

fluid. Phloxine and methylene blue stain was so vigorous in its action that polychromatic effects were obliterated. Delafield's, Harris', or Ehrlich's acid hematoxylin were all satisfactory. The commonly used eosin Y was better than ethyl eosin, in that the latter, like phloxine, was too vigorous in its staining affinities. Feulgen's technic was used to determine whether thymonucleic acid was present in the inclusion bodies and thereby obtain an evaluation of the tinctorial reactions of eosin and Giemsa's stains.

Observations

I. Correlation of Clinical Record with the Gross and Histopathologic Findings

Some information may be gained by the examination of the internal organs of animals brought to the clinic post mortem and of those killed at late stages in the course of the disease. Frequently they showed hyperemia in the wall of the gastrointestinal tract, in small lymph nodes adjacent to the upper colon, and in the cluster of large and small nodes close to the ileocecal junction. Sometimes there was enlargement of the lymph nodes, but not always. From such animals, however, no information was obtained as to the time when lesions developed.

Among the cats used experimentally, nos. 79 to 82 gave the most exact information on the development of the gross, histologic, and cytologic lesions and their relationship to the animal's behavior, weight, temperature, and total and differential leukocyte counts.

The data are too voluminous to be presented as a single unit and, therefore, have been set forth in Tables I, II, and III and also in the description of the histopathogenesis which follows. Table I gives the clinical data.

The clinical records on the cats were begun 3 days after the animals were brought to the laboratory and were continued for 4 days more before inoculation of virus was made. During this period there was considerable variability in weight and total leukocyte count, which probably represents reactions to confinement in cages in a basement room, to manipulations necessary to obtain the data needed, and to the use of several types of dehydrated food. The weights recorded showed such large fluctuations at first that it was difficult to detect any significant loss until about 6 days after inoculation, with the possible exception of cat 81. Loss of weight occurred about the time the animals refused food and vomiting began.

A significant rise in temperature did not develop until 8 days after

TABLE I

Clinical Data on Cats 79 to 82

Date	Weight in gm. Cat no. 80	82	81	79	Rectal temperature Cat no. 80	82	81	79	Total white blood cells per cmm. Cat no. 80	82	81	79
Oct. 11	2210	1850	2445	2425	102.8	102.4	102.2	103.0	9,700	16,175	17,625	11,250
12	2355	1800	2170	2280	102.4	102.2	103.0	102.8	18,800	14,900	13,600	14,575
13	2330	1810	2175	2355	103.2	102.3	102.5	102.4	21,850	16,000	12,000	13,350
14	2090	1720	2285	2240	103.6	102.6	103.4	103.0	9,800	16,100	12,550	8,800
15	2440	1785	2290	2465	102.4	101.3	102.4	101.3	22,100	12,250	15,200	16,950
16	2245	1745	2345	2360	105.0	104.0	104.4	101.4	5,500	9,250	9,550	15,400
17	2285	1720	2135	2260	104.0	103.0	103.0	102.9	4,550	6,150	5,800	12,200
18		1680	2095	2245		102.4	102.0	102.2		4,550	7,600	3,850
19		1750	2065	2320		102.8	103.4	103.2		5,800	6,000	3,950
20		1700	2025	2225		102.3	101.8	100.0		2,300	6,100	4,000
21			1925	2160			103.0	102.5			3,150	2,250
22			1780	2020			106.3	105.3			200	350
23				1965				103.2				1,450
24				1905				101.0				17,200
25				1830				102.0				25,400
26				1780				102.0				13,100

Differential count of leukocytes

Date	Cat no. 80* Neut.	Lym.	Mon.	Eos.	Cat no. 82† Neut.	Lym.	Mon.	Eos.	Cat no. 81* Neut.	Lym.	Mon.	Eos.	Cat no. 79* Neut.	Lym.	Mon.	Eos.
Oct. 11	45	52	2	1	44	42	3	11	43	52	1	4	52	42	3	3
12	44	55	1	0	40	45	2	13	44	48	1	7	53	46	1	0
13	33	67	0	0	48	37	2	13	42	53	3	2	49	45	2	4
14	39	56	1	4	75	21	1	3	34	62	1	3	38	62	0	2
15	40	52	3	5	48	38	1	13	26	72	1	1	44	50	4	2
16	56	37	1	6	59	29	2	14	86	20	4	8	60	36	0	4
17	68	25	0	7	70	22	0	8	36	60	2	2	88	9	1	2
18					48	45	1	6	48	49	0	3	41	49	8	2
19					69	20	0	11	53	43	4	0	44	54	0	2
20					21	73	0	6	47	50	1	2	22	75	1	2
21									6	90	2	2‡	24	70	6	0‖
22									0	95	0	5§	4	48	8	40¶
23													24	48	12	16#
24													84**	15	0	1
25													89**	10	0	1
26													82**	15	2	1

Inoculation of virus was made after the records were obtained on October 14.

* At necropsy there were found one ascarid and one tapeworm in cat 79, four ascarids and one tapeworm in cat 80 and one ascarid in cat 81.

† Cat 82 revealed coccidial oocysts at fecal examination.

Percentages were based on less than 100 cells: ‡50 cells; § 20 cells; ‖ 27 cells; ¶ 25 cells; # 50 cells.

** All parasites were juvenile forms.

inoculation, at which time the leukocyte count was lowest. In cat 79, which survived the crisis, the temperature was normal on the following day.

The drop in total leukocyte count and general trends in the differential count are in close agreement with the reports given in the literature for this disease. The proportion of lymphocytes to neutrophils before inoculation does not agree fully with the averages, and may go beyond the range limits, as given by Ackart, Shaw, and Lawrence,[8] for the normal cat. This may have been due to the new environmental conditions for the animals.

The following descriptions of reactions, when correlated with the data of Tables I to III, give a clear distinction between primary and secondary tissue reactions during the development of the disease and recovery, as well as an evaluation of the necropsy findings.

Cat 80, 3 Days after Inoculation

The behavior, general appearance, and appetite of cat 80, 3 days after inoculation, were normal. All internal organs were normal grossly except the small *lymph nodes* adjacent to the colon. Already there was some hyperemia which gave the appearance of a rosette in several of them. Microscopically, not only the nodes with rosettes but also those which revealed no gross abnormalities were similarly affected. Medullary and cortical sinuses were partially depleted of lymphocytes. More leukocytes were phagocytized in the medullary sinuses than in the corresponding nodes of control cats but this reaction was absent in the cortical sinuses. The erythrocytes were phagocytized in considerable numbers in both cortical and medullary sinuses. They were not disrupted but were ingested intact in as large numbers as it was possible to crowd into the cytoplasm of the macrophage. This was clearly shown after Masson's trichrome stain. The medullary cords were still expanded with lymphocytes. The secondary nodules appeared definitely enlarged but there was no hyalinization such as developed in later stages. The primary nodule appeared about the same as in the normal cats.

Lymph nodules of the terminal ileum showed secondary centers which were not so well defined as in healthy cats, in that the large cells characteristic of this area merged into the primary nodules. No hyalin was present. In the nodules were numerous degenerating eosinophils which were not present in normal cats. Erythrophagocytosis was not evident.

Intranuclear inclusions were present at this time in both the lymph nodes and the lymph nodules of the intestine.

The *colon* showed no changes but the mucosa of the *lower ileum* was affected. No intranuclear inclusions were present but degeneration of the ends of the villi had begun. Beneath the epithelium of the tips a coagulum of serum was conspicuous. The supporting tissue seemed to retract, thus removing the blood supply from the epithelium, and some of the cells already showed autolytic changes in their basal ends. The mucosa of the *duodenum* showed no reaction at this time.

The *spleen* was less reactive than the lymph nodes and nodules. There was extensive disruption, however, of the erythrocytes in the tissue spaces. A few inclusions were present. The only reaction in the *liver* was an increased vacuolization of hepatic cells. Many nuclei in these cells were indented, like red blood cells in hypertonic solution, but were not pyknotic. These changes involved the central

Inoculation of virus was made after the records were obtained on October 14.
* At necropsy there were found one ascarid and one tapeworm in cat 79, four ascarids and one tapeworm in cat 80 and one ascarid in cat 81.
† Cat 82 revealed coccidial oocysts at fecal examination.
Percentages were based on no less than 100 cells: ‡50 cells; §80 cells; ‖27 cells; ¶25 cells; #50 cells.

portions of the liver. In the periphery, the sinusoids were almost completely collapsed and the cells and their nuclei were more nearly normal in appearance.

The *pancreas* at this time showed no change.

The *kidney* showed a few tubules which had an acidophilia characteristic of autolysis but the same change and to about the same degree was present in some tubules of normal cats.

Cat 82, 6 Days after Inoculation

Six days after inoculation, cat 82 showed a loss of appetite but the animal's other reactions did not indicate that it was sick.

Macroscopically, congestion had increased in the *lymph nodes* adjacent to the colon and had spread to include those close to the ileocecal junction. All other organs were normal grossly, including the small intestine. Microscopically, the medullary sinuses of the nodes were depleted to about the same extent as they were at 3 days. The cortical sinuses contained not only a great many macrophages loaded with erythrocytes but also many neutrophils. Many erythrocytes within the macrophages were showing degenerative changes. Phagocytosis of leukocytes was greater than at 3 days. Practically all of the medullary cords were collapsed. The primary nodules were unchanged but the secondary centers contained cells with strongly eosinophilic cytoplasm and after Masson's trichrome stain there were seen droplets of hyaline material among them.

The primary *nodules of the terminal ileum* were not conspicuously changed although they contained more cells with large nuclei than is normal. The eosinophilia and hyalinization of the secondary nodules was similar to that found in the lymph nodes. As in cat 80, no phagocytosis of erythrocytes or leukocytes was found, but there was a conspicuous infiltration of eosinophils, in many of which the granules were coalescing and degenerating.

Intranuclear inclusions were present in both lymph nodes and nodules, and were slightly more abundant at this time (Table III).

The *colon* showed no significant microscopic changes. The *lower ileum* was severely damaged, in that the villi had been reduced to about half their normal length. The mucous cells were depleted and disorganization was beginning in the epithelium of the glandular area. The *duodenum* still lagged behind in extent of tissue destruction although intranuclear inclusions were as numerous here as in the lower ileum.

In the *spleen*, red blood cells did not fill the tissue spaces so abundantly as was found at 3 days; the splenic pulp seemed more closely packed.

All of the nuclei of the *liver* had a spherical shape, the cytoplasm was more vacuolated. Sinusoids and hepatic cords were intact.

In the acini of the *pancreas* the cells were intensely stained and their basal ends sometimes shrank away from the cell wall when fixed.

The *kidney* showed a greater number of tubules with cells with vacuolated cytoplasm and showing autolytic changes than were found in the healthy cats or in cat 80.

Cat 81, 8 Days after Inoculation (Height of the Disease)

Cat 81 was killed at the end of the eighth day, instead of the ninth as originally planned, because it probably would not have survived the night. It was too weak to stand. Hyperemia was widespread in the lymph nodes along the colon and ileum. The walls of the small intestine showed extensive areas of congestion except in the region of the duodenum. The same reaction was visible also in the spleen and in the uterine tubes. Fat was yellowish and not so abundant as in cats 80 and 82. Other visceral organs appeared normal grossly. Microscopically, the cortical and

medullary sinuses of the *lymph nodes* adjacent to the colon were fairly well filled with cells, more so than they were earlier in the disease. Those in the lymph nodes adjacent to the ileocecal junction were entirely depleted so that the meshwork of reticulocytes was clearly shown. Phagocytosis of erythrocytes was still abundant in a node by the colon but was less in an ileocecal node. In both, the picture of phagocytosis had changed, in that very few erythrocytes within the macrophages were intact but had disintegrated and the cytoplasm of the macrophages had frayed out in many cases. The blood plasm contained much cell débris. This reaction at the terminal stage of the disease was noted by Hammon and Enders.[2] The medullary cords showed varying degrees of collapse and expansion. The centers of all nodules were strongly hyaline but some showed early stages of regression. Few cells were contained within the hyaline area. The large cells characteristic of the normal secondary nodule lay at the periphery of the hyaline mass and beyond them the character of the primary nodule was essentially the same as before.

The hyaline areas in the *lymph nodules in the terminal ileum* showed early stages in regression. Beyond the centers there was a more or less uniform mingling of large and small cell types. There was no phagocytosis of blood cells. Degenerating eosinophils were present but less abundant than in cats 80 and 82. The intranuclear inclusions had decreased to about the number found at 3 days.

The *colon* even at this stage showed little reaction to the disease beyond some loss of mucus from the goblet cells at the base of the glands and greater loss of epithelial cells. This agrees with the observations of Lawrence *et al.*,[1] but the damage to the *lower ileum* was even more extensive than before, in that the villi were reduced to short knobs covered with low columnar epithelium. Many cells were lost from Lieberkühn's crypts, and those remaining had compensated by changing to a squamous shape. Mucous cells were practically gone. The *duodenum* for the first time showed extensive destruction of villi, including the epithelium of Lieberkühn's crypts. Fewer intranuclear inclusions were present in the duodenum than in cat 82 but in the lower ileum they were about as numerous as they were 3 days earlier.

The hepatic cells of the *liver* now showed no vacuoles and the cytoplasm was densely granular. Much of the cytoplasm was torn away, leaving frayed cells with nuclei at the margin. The nucleoli were small. The sinusoids were filled with coagulum and there was slight perivascular infiltration around some vessels.

The shrinkage of acinar cells in the *pancreas* was greater than in cat 80.

The *kidney* showed a large number of damaged tubules, represented by cytoplasmic vacuolization in some cases and by autolytic eosinophilia in others.

Cat 79, 12 Days after Inoculation (3 Days after the Crisis)

Twelve days after inoculation, cat 79 showed increased alertness and activity over that shown when the leukocyte count was lowest. However, the animal still did not eat and there was continued loss of weight. It is doubtful whether it would have recovered ultimately. Grossly, neither lymph nodes nor small intestine showed hyperemia. The other internal organs appeared normal except that the gallbladder was greatly distended with bile. This was not due to blockage of the duct since it was possible to milk the bile into the duodenum with the handle of the scalpel. The cortical and medullary sinuses of the *mesenteric lymph nodes* were filled with cells and closely resembled the normal condition, both as to cell types and distribution. Only a few erythrocytes were ingested and the stages were all late in the process. No ingested leukocytes were seen. There was wide variation in the filling of the medullary cords, ranging from empty to full. The nodules of the node had changed markedly. All hyalin was gone and it had been replaced by aggregations of small lymphocytes which were surrounded by larger lymphocytes having the general

appearance of cell types found in a normal secondary center. These, in turn, were surrounded by a mixture of cell types characteristic of the cortex.

Hyalin had gone from some of the *lymph nodules of the terminal ileum* and was about half regressed in others. The stellate appearance of the hyalin suggested that regression occurs from the periphery by the invasion of small lymphocytes. Cells peripheral to the small lymphocytes showed a uniform mingling of large and small types. No phagocytosis of erythrocytes or leukocytes was seen and there were no eosinophils.

The *colon* was still unchanged. The mucosa of the lower *ileum* showed improvement over that found at the crisis of the disease. Mucous cells were more numerous. The villi were still lower but better covered with epithelium. That these changes represent improvement and not a less critical illness is indicated by the regression of hyalin in the lymph nodules of this tissue. The *duodenum* showed little or no improvement except that the epithelium covering the villi was more nearly normal than in cat 81.

In the *spleen,* hyaline areas in the nodules had almost entirely regressed. Débris of previously ruptured erythrocytes was still present. One giant cell was seen, but no intranuclear inclusions.

The *liver* also showed a little improvement, in that some vacuolization of the cytoplasm of the hepatic cells had returned. In the sinusoids were coagulum, neutrophils and other reacting cells.

The *pancreas* revealed a greater disruption of acinar organization than was found at the crisis of the disease. There was leukocytic invasion.

The *kidney* also showed some recovery from the condition found at the crisis, in that the tissue disorganization was less and the striated cuticular borders were more definite.

Clinic Cats

In the group of 12 cats from the clinic, the medullary and cortical sinuses of the *mesenteric lymph nodes* varied from those filled with phagocytic macrophages to those which had a mixture of phagocytes and normal lymphoid cells. The medullary cords varied from collapsed to full. Hyaline centers were usually present in the nodules but in many examples they showed varying degrees of regression and in some they had gone completely. The primary nodules were not particularly reactive, except in one case where there was marked loss of cells; in others the organization of the primary nodule was more normal than in cat 79. Intranuclear inclusions were found in the lymph node in one clinic case.

Practically all of the clinic cats showed greater disorganization of the *lymph nodules in the terminal ileum* than did the experimental cats. The lymphoid cells were diffusely scattered and all traces of organization into primary and secondary nodules were gone. Some showed areas of necrosis and neutrophilic infiltration, not found in the experimental cats. A few clinic cats were less severely injured in that the nodules showed hyaline centers. Eosinophils were observed in only one case.

The mucosa of the *colon* in 4 clinic cats was half eroded but post-mortem changes may have played a large part in this. The material collected in the clinic has been of little value in studying the destruction due to disease in the intestinal tract because post-mortem changes were also present. In the *lower ileum* the villi were sometimes mere stubs of connective tissue without a trace of epithelium covering them. In most cases, practically all of the glandular epithelium was destroyed also. Material from the *duodenum* was not taken from these animals.

The *spleen* showed varying degrees of hyalinization; in some it was conspicuous and in others it had entirely regressed. Inclusions were present in one case.

The *liver* damage was in nearly every case more severe in the clinic animals than in the experimental ones. In some, the hepatic cords became dissociated into isolated cells, and the sinusoids were disrupted and often collapsed. The cytoplasm was

dense and darkly staining. In some livers there was nuclear hypertrophy with aggregations of oxychromatin around the nucleolus somewhat similar to Figure 1. In one clinic cat, stages of recovery had set in: infiltration of phagocytic cells, decreased staining of cytoplasm, and a tendency to form numerous basichromatic granules in the nuclei. There were numerous focal areas of reorganization. In one of these there was found an intranuclear inclusion of the homogeneous type.

The *pancreas* was taken in only one animal. There was extensive acinar disorganization accompanied by leukocytic infiltration.

The *kidneys,* like the other tissues from clinic animals, showed a great variability. None were more nearly normal than cat 81 and most of them revealed disorganization with degeneration of the tubule cells.

Certain conclusions seem warranted on the basis of the histologic changes during the development of the disease. The lymphoid tissue is the first to react. There is a draining off of available lymphoid cells from the stroma of the lymph nodes, later followed by depletion of the cords, but the nodules of the cortex remain intact throughout the disease. The nodules react by changes in organization, develop hyalin in the center, but quickly return to normal after the crisis has passed.

The phagocytosis of erythrocytes is a reaction which begins early in the disease. The ingested cells in the early phases are normal in appearance. The reaction is still strong at the sixth day although disintegration of previously ingested material is apparent. By the time the crisis is reached there is no longer ingestion of fresh erythrocytes, only the completion of the process of disintegration. These facts may indicate, possibly, that the virus is disseminated by adherence to the surface of the red blood cells, or simply that early in the disease there has been hemorrhage into the lymph channels. Macchiavello and Bezerra Coutinho [9] noted that the blood was the first tissue to lose its infectivity.

Since this disease depletes the circulatory leukocytes so severely, it might be expected that there would be severe phagocytosis of these cells. However, there is no marked phagocytic action against the leukocytes in the early phases of the disease. It reaches a peak about the sixth day, but at the time of the crisis and in the recovery period the leukocytes ingested are few or none. The number of leukocytes in proportion to the erythrocytes is so small and the reaction against the erythrocytes is so vigorous that significant phagocytosis of the leukocytes may have been obscured. It has been suggested that they may be lost due to a leukotoxic substance and by way of the intestinal lumen.[10] Degeneration of the eosinophils was found only in the lymph nodules of the lower ileum. These cells may be associated with parasitism and may have no relationship to the action of the virus. On the other hand, there seemed to be a cycle of eosinophilic reaction in the three cats which had the same parasites, and the cells are usually absent in cats seen at the clinic.

The mucosa of the ileum is the second tissue to react and thence the reaction progresses to the duodenum. The stomach and esophagus were not examined.

The reaction in the spleen is limited mostly to the lymphoid areas. The liver, pancreas, and kidney are the last visceral tissues to respond to the development of the virus and they show their greatest damage when the disease is at its height or even after the crisis has passed.

The destructive action on the liver may be even greater than in the lymph nodes but there is nothing to indicate that it is the primary focus of action for the virus. In view of the extensive liver damage, it is interesting that Kikuth, Gönnert, and Schweickert tried a vitamin C diet in the treatment of this disease. They obtained negative results.[5] Apparent success of vitamin C as a clinical therapeutic measure in combination with sucrose has been experienced by Riser.[11]

The reactions of the animal do not become those of a "sick cat" until late in the disease when the macroscopic changes of the internal organs are far advanced. Thus, in dealing with the disease clinically, it is possible that a cat may not be brought to the attention of a veterinarian until the blood count has returned to normal. In that case, at the time of death, diagnostic gross, histologic, and cytologic criteria have disappeared, except for the clue which may come from the presence of juvenile forms in the circulating blood.

II. CELLULAR ALTERATIONS

The intranuclear inclusions described when the agent of the disease was first identified as a virus have been the principal subject of this investigation. It has been determined how many different types occur, their relative number at different phases of the disease, their distribution in the various tissues, the cycle through which they pass from young to old stages, and finally a comparison with other known inclusions.

Two types of inclusions have been discovered in this material, granular and homogeneous. The granular type is further divisible into diffuse granular and clustered granular.

Clustered Granular Intranuclear Inclusions

The clustered granular intranuclear inclusion is the most commonly found type and is reported by Hammon and Enders,[2] by Lawrence *et al.*,[1] * and by Kikuth, Gönnert, and Schweickert.[5] In its fully de-

* A comparison of inclusions in our material with that reported at the University of Rochester Medical School was possible through the courtesy of Dr. Jerome T. Syverton who sent us six stained slides.

veloped form it is morphologically identical with inclusions of yellow fever as described by Cowdry and Kitchen [12] and on the basis of structure should be classified in the same group as yellow fever. However, in the cycle of changes which involve early and late phases, it is readily distinguishable from the inclusions of yellow fever and under certain conditions may resemble those of herpes.

As with all studies on the cycle of inclusion formation, the early stages are difficult to find and to distinguish from the nuclear variations common to any reacting tissue. The most reliable criterion that a nucleus is developing toward inclusion formation is the pulling away of the normal reticulum, which is usually somewhat basophilic, toward the nuclear membrane. This reaction in itself is not distinctive, in that it occurs frequently in nuclei under various abnormal conditions, but if an inclusion is developing, strands of linin network lag behind on which there may be a few oxychromatic granules at the interstices (Fig. 2). These granules are lightly staining, are colored only by the acid dyes and are Feulgen negative. Sometimes they are not distinguishable as discrete granules but appear to be a lightly colored, homogeneous mass (Fig. 3). This may resemble a plasmosome of an amphinucleolus in that basichromatic granules are sometimes associated with it, but the color reaction in our preparations is that characteristic of an inclusion and not a plasmosome.* Even in these acidophilic masses a suggestion of granular structure may be seen, if an optical system of maximum efficiency is employed (see footnote, page 457).

These early stages differ somewhat from those found by Cowdry and Kitchen [12] for yellow fever. In yellow fever the first granules to appear have already separated from acidophilic linin fibers and are located in the center of the halo, but in panleukopenia of cats no early stage was found having only a few discrete granules which were lying free from linin fibers in the center of a halo, although a thorough search was made for them. Additional evidence that in this disease the earliest granules are associated with an acidophilic linin network is derived from a study of the diffuse granular inclusion to be described later.

Frequently, nuclei are found in which there are accumulations of discrete granules clustered near the amphinucleolus (Fig. 1). This

* Usually, plasmosomes observed in this material (if a stain giving a good range of tinctorial effects is used) are neutrophilic (Figs. 1, 3, and 14). The term "neutrophilic" is used here to denote a gray color which is due to the mixing of acidophilic and basophilic substances in the same structure. The gray color is a result of the blue of hematoxylin mingled with the orange of eosin. The same result occurs when the red of Feulgen's stain is blended with fast green as a counterstain. In an earlier paper an inclusion showing a similar reaction was called a "dark body" or an acidobasophilic inclusion.[13]

might be suggestive of an early stage except that the generalized reaction toward margination is lacking. Moreover, it is a type of reaction frequently found in nuclei under abnormal stimulation when no inclusion bodies are developed.

Once the early stage has passed, the subsequent stages are clearly distinctive. The granules become more numerous and are of uniform size, about one-third to one-half of a micron. The staining reaction is still eosinophilic with no basophilia (Fig. 4). A few delicate, radiating strands extend from the inclusion to the nuclear membrane. Concomitant with the development of the inclusion body is a completion of margination except for the nucleolus (Figs. 4 and 5). The nucleolus lags behind and is sometimes stretched across the nucleus as a darkly staining band, as shown by Kikuth, Gönnert, and Schweickert.[5] The lagging of the nucleolus is characteristic of yellow fever also and was used by Cowdry and Kitchen [12] as a distinguishing feature between that disease and herpes.

A fully formed inclusion may show a clear orange color with eosin but more often its tinctorial reaction changes toward a superimposed basophilia. The granules become more numerous and closely packed (Fig. 5). The radiating fibers may be few or none. It is suspected that they are present even when invisible because the inclusion tends to have irregularities in its contour which are associated with radiating linin fibers when these can be detected.

When basophilia * has developed in the inclusion body, it is regarded as having passed its ascendency and is now entering its terminal stages. The inclusion becomes more compact, basophilia increases, and the nucleolus tends to marginate at this time if it has not already done so.

Ultimately the inclusion becomes homogeneous with a well developed halo and very few, if any, radiating strands (Figs. 6 and 7). It is impossible to see granules in the homogeneous stage but they can be distinguished in all of the phases which lead up to it. Morphologically, it resembles an isolated plasmosome but it is usually distinguishable by the staining reaction in that it has a mauve color after hematoxylin and eosin, whereas the plasmosome is usually neutrophilic. In Figure 7 is shown both an isolated plasmosome and an inclusion body. The cell shown in Figure 14 has a typical isolated plasmosome. Not only is the tinctorial reaction of the particular cell shown in Figure 14 distinctive

* The term "basophilia" will be used to mean that a slight amount of basophilic substance is present so that the color reaction of the eosin has changed from a coral pink to a mauve or magenta. The term has not been used to mean that a structure has taken on the same staining reactions as chromatin.

enough to separate it from a late granular inclusion, but a late inclusion would not be found associated with such an early phase of nuclear margination.

Diffuse Granular Inclusion

The diffuse granular intranuclear inclusion is less common than the clustered granular inclusion. The ratio may vary from 1:5 to 1:50 (Tables II and III). Since at least several hundred inclusions should be examined critically in order to arrive at any opinion concerning their course of development, it has been a handicap that diffuse granular inclusions were relatively so few. However, their morphologic configuration is sufficiently definite in the fully formed condition to classify them as a distinct type. Margination of chromatin is more vigorous in the early phases of this type than in the clustered granular type but during the margination there remains behind a more extensive and more conspicuous acidophilic reticulum. On this reticulum, at the interstices, are scattered eosinophilic granules. They have a tinctorial reaction identical with the reticulum and are not refractile so that it is sometimes difficult to see the granules in the early stages (Figs. 8 and 9). Figure 8 is quite similar to Figure 2 but the following criteria were used to distinguish them. In the early clustered type the linin fibers radiating from the granules may be slightly basophilic whereas in the diffuse type they are clearly eosinophilic throughout. In the former the granules are more discrete and independent of the reticulum and in the latter they are hardly more than enlargements at the crossing of the fibers.

As the diffuse granular inclusion develops, the acidophilic reticulum increases in amount and so do the granules, but they remain located at the interstices as in the early stages and still do not develop refractile properties (Figs. 9 and 10) as do the clustered inclusions.

The chromatin and nucleolus react differently in the two types also, in that complete margination comes relatively earlier in the diffuse granular type and the nucleolus never remains in the center of the nucleus. In most cases it has disappeared entirely by the time the inclusion is fully formed.

The inclusion, therefore, is composed of granules plus reticulum and the reticulum has many radiating strands which extend to the nuclear membrane. As a result, the halo is less clearly defined than in the clustered type. In some examples the reticulum fills nearly all of the nucleus.

The late stages are subject to the same limitations and doubts as are the early stages because the examples are so few. The inclusions develop a superimposed basophilia and the linin network contracts

somewhat (Fig. 11). Since the granules still remain attached to the reticulum, the contraction of the network produces small vacuolar spaces. As contraction and increased basophilia continue, a stage is reached comparable to the homogeneous phases of the clustered granular inclusion except that the vacuolization is retained (Fig. 12). Further evidence that such an inclusion is derived from a diffuse type is given by the large number of radiating fibers in contrast to the small amount of reticulum and few radiating fibers of the clustered type.

No morphologic or developmental differences whatever have been found between the diffuse and clustered granular inclusions which appear in the lymph glands and those in the intestinal epithelium. Nor do the clustered granular inclusions develop in one cell type and the diffuse inclusions in another. Since they are found together and in similar cells, the difference in reaction is probably a physiologic one. This idea is supported by the fact that sometimes a young, diffuse inclusion may have at one side a group of granules characteristic of the clustered type (Fig. 13). In spite of the fact that structures characteristic of both types may occasionally be present in the same nucleus, yet it is our opinion that one is not a developmental stage of the other but that they represent two variations in nuclear reaction to the disease stimulus and that each inclusion type follows its own cycle of ageing.

The time involved in the inclusion cycle could not be learned from the data available in this study, except that the inclusion develops and disappears quickly and in this respect is similar to the monocytic reaction in the virus of the submaxillary gland of guinea-pigs.[13]

Homogeneous Inclusions

In this material the fully formed homogeneous inclusion, not of the type derived from a granular inclusion but from a plasmosome, as will be shown later, is usually neutrophilic with eosin Y (Figs. 14 and 25), but may be stained red with ethyl eosin or phloxine. It lies in the center of a halo, has few and usually no radiating strands, and its edge may or may not be slightly refractile. The chromatin may be partially marginated, as in Figures 14 and 25, or completely marginated, as in Figures 15, 16, and 26.

The developmental stages were most clearly demonstrated in the liver of cat 82. The inclusion is derived from a plasmosome portion of an amphinucleolus. In the cell type from which it develops (Fig. 23) the plasmosome is surrounded by basichromatin granules. The reticulum is normal and granules are scattered between the nuclear membrane and the nucleolus. Practically every nucleus in the liver has this

structure, but occasionally in sections of liver there are groups of cells in which marginating chromatin may be found leaving the plasmosome (Fig. 24). In Figure 25 the process has progressed far enough so that the plasmosome would probably be called a homogeneous intranuclear inclusion and would be classed as Cowdry's type B.[14] Complete margination was not found in the liver cells but was observed in the lymphoid tissue and intestinal epithelium (Figs. 15, 16, and 26). A similar derivation of an inclusion from a plasmosome was described by Birch and Lucas [15] as a response to subcutaneous injection of aluminum oxide in guinea-pigs, except that in that case the plasmosome gave an acidophilic reaction. In the cat, panleukopenia may produce the same acidophilic reaction in some cells (Fig. 15) although the usual reaction has been neutrophilic. Figure 15 appears almost identical with an early stage in the development of intranuclear inclusions by the virus of the submaxillary gland of guinea-pigs in which homogeneous eosinophilic and neutrophilic inclusions were in the same nucleus (Fig. 5 in paper by Rosenbusch and Lucas [13]). It was concluded by Rosenbusch and Lucas that the difference in staining probably represented difference in age, the younger being more acidophilic and the older more neutrophilic. The same conclusion seems valid for reactions such as shown in Figure 15. Sometimes a mixture of materials may be found in which basophilic masses are mixed with acidophilic substance (Fig. 16), or they may be so intimately mingled (Fig. 17) that it is impossible to determine whether the inclusion was derived by way of a plasmosome or a granular inclusion.

Other peculiar reactions of the plasmosome have occasionally been observed. One of these is an exaggeration of vacuolization. Vacuolization in itself is a common reaction but only occasionally does it go as far as shown in Figure 26.

Even in the prophase of mitosis, a plasmosome can look like a typical homogeneous inclusion (Fig. 29-b). Upon superficial examination the plasmosome inclusion (in this case, double) seems to be surrounded by a halo and the chromatin to be marginated. The chromatin is actually marginated but it is assembled in the form of a spireme, as may be seen by examining the nuclear sphere at all levels of focus (Fig. 29-a). By a study of earlier prophases, it was determined that isolation of the plasmosome and margination was the normal procedure in mitosis in this material (Figs. 27 and 28). In Figure 27 the chromatin makes many contacts with the plasmosome, as it does in the resting stage, but as the spireme thread is organized, it gradually pulls away from the plasmosome (Figs. 28 and 29).

Mitosis and Intranuclear Inclusions

Mitosis in cells which contain cytoplasmic inclusions of virus origin is well known.[16, 17] Ivanovics and Hyde [18] described lobulation and amitosis associated with virus III in tissue culture but no instance of true mitosis has been recorded in cells which bore a granular intranuclear inclusion.*

The intestinal epithelium is particularly favorable material for study in that the rate of mitosis is higher than in most tissues of the body. Although diligent search was made among many hundreds of inclusion-bearing cells, only two examples were found (Figs. 18 and 19). Figure 18 is a prophase spireme and shows a clustered granular inclusion in about the middle phase of development. The spireme threads are fairly well organized at the left side of the nucleus but poorly so on the right side. Figure 19 is not a normal mitosis and perhaps represents a late prophase or an abortive metaphase. It is doubtful that mitosis would be successfully completed. It would be interesting to know, because of its bearing on cytogenetic problems, whether the failure is due to mechanical causes or to deprivation of oxychromatin.

Cellular Death and the Inclusion Body

It has been maintained in previous publications [13, 15, 19] that the development of an inclusion body does not in itself predestine the death of the cell. Thus, death, when it comes to inclusion-bearing cells, is a result of the environmental stimuli which are set up by the action of a virus or other foreign agent. This conclusion is borne out in the present study, in that lethal changes may be superimposed at any phase of the development of an inclusion body. In Figure 20, necrosis and shrinkage of the nucleus have come at a time when the inclusion is at a phase of development comparable to that shown in Figures 4 and 5. In Figures 21 and 22 that change has come at later phases. Nuclear death has progressed farther in Figure 22 than in Figure 21 in that karyorrhexis has begun, as evidenced by the isolated chromatin granules and invisibility of nuclear membrane. In the lower cell of Figure 22 the chromatin has liquefied. This cell may have had an inclusion body, judging by the position of the plasmosome, but there are no other criteria to determine the point.

* Since this was written, two reports of mitosis in nuclei which contained inclusions have come to our attention. One concerned a homogenous type associated with thyroid tumors (Stewart, C. F. Intranuclear inclusion bodies in carcinoma of the thyroid gland. *Am. J. Cancer*, 1939, 37, 196–200). It would be interesting to know whether these inclusion bodies had origin from plasmosomes. The second describes a peculiar homogenous body found in renal nuclei following administration of bismuth (Pappenheimer, A. M., and Maechling, E. H. Inclusions in renal epithelial cells following the use of certain bismuth preparations. *Am. J. Path.*, 1934, 10, 577–588).

TABLE II

Mitosis and Inclusions in the Epithelium of the Lower Ileum

Cat no.	Days after inoculation	Time killed	Total cells counted	Mitotic figures		Clustered granular inclusions		Diffuse granular inclusions		Ratio of diffuse to clustered inclusions	Empty nuclei	
				No.	Per cent	No.	Per cent	No.	Per cent		No.	Per cent
77	Normal cat	2:30 p.m.	10,000	111	1.11	0	—	0	—	—	0	—
78	Normal cat	4:00 p.m.	10,000	99	0.99	0	—	0	—	—	0	—
33	In extremis	11:00 a.m.	10,026	49	0.49	661	6.59	77	0.77	1:8.6	13	0.13
80	3 days	8:30 p.m.	10,024	116	1.16	0	—	0	—	—	0	—
82	6 days	2:45 p.m.	7,081	102	1.44	175	2.47	8	0.11	1:22.4	1	0.014
81	8 days (crisis)	10:00 p.m.	2,725	31	1.14	81	2.97	9	0.33	1:9.0	2	0.073
79	12 days	12:15 p.m.	1,0030	90	0.90	2	0.02	0	—	—	0	—

Relative Number of Inclusion-bearing Cells and Mitoses during the Course of the Disease

It was the original purpose of this investigation to determine to what extent the inclusion bodies could be used in clinical diagnosis. It was soon discovered that they had less value than originally hoped because at the death of the animal they sometimes were present but usually were absent. The cause for this variability was discovered when cats were killed during development and recovery stages of the disease.

Counts were made on the number of mitotic figures, the number of diffuse and clustered granular inclusions, and isolated plasmosomes. As to plasmosomes, only those were included which resembled the ones shown in Figures 14, 15, 16, and 26, and not those which arose as part of the mitotic process (Figs. 28 and 29). The counts are summarized in Tables II and III.

Inclusions first appeared in the lymphoid tissue, not only in the nodes adjacent to the colon where the first congestion of blood vessels was observed, but also in the lymphoid nodules of the lower ileum. By the sixth day after inoculation, up to the time of lowest leukocyte count, the inclusions were present in both lymphoid tissue and intestinal epithelium. They might be found throughout the small intestine, including the duodenum. They were absent from liver, pancreas, kidney, and adrenal during the full course of the disease but a few were present in the spleen. In the

lymph nodes the greatest number of inclusions were found on the sixth day and in the intestinal epithelium the greatest number were present at the height of the disease. This agrees also with observations on the histopathology of the disease. Three days after the crisis had passed the inclusions had disappeared. Only two were found in numerous sections of lymph nodes and intestine examined. Thus, it is evident that the inclusions disappeared soon after the crisis was passed. Support for this idea comes from the examination of clinic cats; only 1 of the 12 showed any inclusions. It is for this reason that it is claimed that negative findings at necropsy have relatively little value in the determination of the cause of death. On the other hand, a positive finding is highly specific.

TABLE III

Occurrence of Inclusions in the Lymph Nodes Adjacent to the Colon

Cat. no.	Hypertrophied cells counted	Clustered granular inclusions		Diffuse granular inclusions		Ratio, diffuse to clustered	Isolated plasmosomes		Empty nuclei	
		No.	Per cent	No.	Per cent		No.	Per cent	No.	Per cent
80	1005	49	4.88	1	0.099	1:49	0	—	0	—
82	1000	60	6.0	4	0.40	1:15.0	2	0.20	6	0.60
81	917	28	3.05	6	0.65	1:4.7	0	—	0	—
79	1000	0	—	0	—	—	0	—	0	—

Empty or ghost nuclei (Fig. 30) were recorded in the counts because they are abundant in yellow fever material and following severe burns. In panleukopenia of cats they are almost completely absent and none of them show the enormous hypertrophy described by Cowdry and Kitchen [12] for yellow fever.

Plasmosome nucleoli which are free from all basichromatin and lie in a halo are also rare and have little significance except to indicate that a nucleus may react in other ways than by the development of an inclusion body.

It was anticipated that the extensive destruction of epithelium during the development of the disease and its rapid repair afterward would be reflected in a change in mitotic rate. Counts of mitoses were made in the region of the glands and did not include the villi. It was discovered that the mitotic rate is remarkably constant and the values obtained differ but little from those found in the normal cats. Since periodicity of mitosis has been reported in several publications,[20-23] it was unfortunate that the animals were not killed at the same hour in the day. It should be emphasized that casual examination of the slide may give an erroneous impression. Before the counts were made, it seemed cer-

tain that cat 79 had many more mitotic figures in the field than the others.

Cat 33 was included in Table II because it had more inclusions than any other animal studied. The data are relatively meager. It was one of the earliest cats used experimentally and was destroyed in a moribund condition 3 days after intraperitoneal injection of 2 cc. of blood from a diseased cat. When killed it had a leukocyte count of 1800 per cmm. and a temperature of 103.0° F. The cat may have been infected spontaneously sometime before the inoculation was given.

Comparison of Inclusions of Panleukopenia of Cats with Those of Herpes, B-Virus, Yellow Fever, Severe Burns, and with Other Granular Types

If studies on the morphology of intranuclear inclusions are going to be of practical value to the pathologist in his diagnosis of disease, it is necessary that distinguishing differences be set forth clearly. Cowdry and Kitchen [12] observed that the nucleolus is not so readily marginated in yellow fever as in herpes simplex and that there is a slight basophilia of herpes inclusions which is absent in those of yellow fever.

The developmental stages of the inclusions in herpes and B-virus,* as well as some additional facts not reported in the literature on the morphology of later phases, are given since they also are valuable in distinguishing inclusion types. The earliest stage which can be identified is not granular but is represented by one or more homogeneous or amorphous masses which are not refractile. The masses are acidophilic. At the time of their appearance the chromatin may not be completely marginated (Fig. 31). There is no difference between these masses and those which frequently appear in nuclei under stimulation as droplets of oxychromatin. In fact, the two are probably identical. Later, there develops, in association with these masses, uniformly fine acidophilic granules (Fig. 32 and lower left cell of Fig. 34). It has been impossible thus far to determine whether the granules arise within the homogeneous masses or invade the cell, as has been maintained by Nicolau and Kopciowska.[24] As the granules increase in number they become evenly spaced and it is possible to distinguish a delicate reticulum joining them (Figs. 33 and 34). This reticulum has not been previously reported but is clearly visible if the granules are far enough apart. It has the same acidophilic reactions as the granules. The halo which is present only in the early phases of granule formation seems to last but

* The senior author is indebted to Dr. A. B. Sabin, Medical School, University of Cincinnati, for help received and facilities made available in his laboratory during several days spent there while collecting and centrifuging tissues from rabbits inoculated with B-virus and pseudorabies.

a short time, so that in many nuclei the granules completely fill the nucleus.

The granules of herpes and B-virus are slightly basophilic and Feulgen positive, especially in the later phases. Two explanations might be given to account for this basophilia: (1) that the granules are the virus agent and, like the inclusions and elementary bodies of varicella, are Feulgen positive; [25] or (2) that herpes and B-virus are so destructive to the cell that some chromatin liquefies, as it always does in autolysis,[19] and thus imparts to the granules their basophilia. The latter explanation certainly applies to the development of basophilia in inclusions of panleukopenia of cats, and when shrinkage of the nuclear membrane has occurred with loss of the halo the resulting color reactions and morphologic features could not be distinguished in any way from those of a herpetic inclusion. If Figures 33 and 34 had been reproduced in color, they would show the same colors as do Figures 21 and 22. If herpetic granules represent the organism, it might be expected that diploid forms would be discovered. Thus far they have not been observed.

A careful comparison of herpes (Figs. 31, 32, and 33) with B-virus (Fig. 34) has failed to reveal any morphologic or tinctorial differences between the inclusions produced by the two viruses, yet immunologic differences have been established.[26]

A comparison of the inclusions of yellow fever and those found after severe burns was made.* The fully formed inclusion was easily identified in the material from patients who had been burned (Figs. 37, 38, and 39). Early stages may, perhaps, be represented by nuclear reactions of the type shown in Figures 35 and 36, but on the other hand they may represent merely a diffuse hyperoxychromatic reaction which does not lead to typical inclusion formation. It was clearly evident, even with the limited amount of material, that all nuclear reactions do not lead to granular inclusion formation. Sometimes the stimulus produced one to six homogeneous spherical masses in one nucleus, and other "atypical" changes. This variability is reminiscent of some controversies in the literature on yellow fever as to significance of the presence or absence of typical or atypical acidophilic inclusions. The empty nucleus (Fig. 40) is as typical of this material as it is of yellow fever. The structure, apparent developmental cycle, and tinctorial reactions of the inclusions from fatal burns and yellow fever have a

* One of us (A.M.L.) is indebted to Dr. William Boyd of Toronto who kindly sent slides and a block of liver tissue which showed inclusions from cases of burns in man. From some of this material the publication of Dr. Thomas H. Belt [28] was prepared. These were compared with slides of yellow fever in the monkey sent by Dr. E. V. Cowdry of St. Louis.

very close similarity to each other; more so than either of them have to those of panleukopenia of cats or to the inclusions in the Guatemalan amazon,[27] in that neither become basophilic or coalesce into homogeneous masses in their late phases. These observations support the conclusions of Belt [28] that the nuclear inclusions of yellow fever and burns are alike.

Both panleukopenia and the kidney disease of the Guatemalan amazon [27] develop homogeneous masses in the late stages but do it in different ways. The former has already been fully described; in the latter, all of the granules do not coalesce at one time but instead form one or several homogeneous masses embedded in the surrounding granules (Figs. 12 and 13 in the publication by Cowdry, Lucas, and Fox [27]). The material was not adequate to determine whether all of the granules and masses may finally coalesce into one large homogeneous body.

The few comparisons given are enough to indicate that a careful cytologic study of inclusion formation in all of the virus diseases would probably bring out individual differences which might well have some diagnostic value.

Discussion and Conclusions

The group of diseases which morphologically belong to the yellow fever type include, in addition to those already mentioned, Rift Valley fever [29] and perhaps Pacheco's disease of parrots and parakeets. There are probably others if their structure and developmental phases were more exactly known.

The diseases which have been included thus far have one factor in common, and this applies to burns as well: they all tend to dehydrate the animal severely. One preliminary experiment was undertaken to determine if dehydration of a healthy cat, by not giving food or water and with frequent doses of ipecac, would produce intranuclear inclusions in the digestive tract. In this single experiment the results were negative, but it should be repeated with a technic which would give more vigorous dehydration of the posterior part of the ileum.

The fact that severe burns will produce intranuclear inclusions nearly identical with those of yellow fever is strong indirect evidence that the virus-produced inclusion is also an oxychromatic segregation. But this does not preclude the possibility that the virus, if present, might be adherent to the oxychromatin granules or embedded within them. The early stages in the formation of inclusion bodies of panleukopenia and other yellow fever types also support the idea of an oxychromatic origin. The later basophilia which may develop in panleukopenia does

not invalidate the idea, because it is fairly certain in this case, as with the submaxillary gland disease of guinea-pigs and ground moles,[30, 31] that it is derived from a solution of basichromatin. Whether basichromatin is the only source of the basophilia in the herpes group of inclusions is still an open question.

Definition of an Inclusion Body

In reviewing the subject of the plasmosome as it reacts in panleukopenia of cats the question is raised, "When is it properly a plasmosome and when a *bona fide* inclusion?" Certainly, structures and nuclear reactions identical with those shown in Figures 14, 15, and 25 have been called inclusion bodies characteristic of virus disease. In panleukopenia of cats, however, they are a minor by-product of plasmosome activity. Also, it has been found in this study that the plasmosome certainly plays no part in the formation of the granular inclusions. It is pushed aside with the basichromatin, and we have concluded, as did Cowdry and Kitchen [12] in yellow fever, that the inclusions which are specific for panleukopenia of cats are not derived from the plasmosome. The question of terminology can probably be answered best in this way: the term, inclusion body, should be applied to those morphologic abnormalities in the cell which, when their complete cycle has been worked out, are found to have a specific or relatively specific association with the agent or pathologic condition which produces them. In panleukopenia, therefore, the plasmosome reactions could not properly be called intranuclear inclusions, but in the guinea-pig after subcutaneous injection of inorganic substances,[15, 32, 33] the same reactions are sufficiently specific for the plasmosome to be called an inclusion body. Likewise, the greatly hypertrophied amphinucleolus which Findlay [34] observed in the livers of a Claxton strain of mice would be considered an intranuclear inclusion although the identical reaction which can occasionally be observed in a normal animal would not be so classified.

This concept of an inclusion body eliminates the difficulties which arise when one defines an intranuclear inclusion as an acidophilic body surrounded by a halo and accompanied by margination of chromatin. It is true that most inclusions which have been described have these characteristics, and it is a useful "rule-of-thumb" to follow, but an analysis of all developmental stages often reveals that these criteria are sometimes applicable only at certain phases of the cycle. Frequently inclusions may be neutrophilic or even fully basophilic at some phase of their development, as are those in fox encephalitis and those described by DeMonbreun and Goodpasture [35] in oral papillomatosis of dogs and as noted by Cowdry, Lucas, and Fox [27] in some kidneys from wild birds and mammals. In some virus diseases the basichromatin does not re-

main marginated against the nuclear membrane but returns to join the surface of the inclusion body. This is true, for example, in late stages in the submaxillary gland disease of guinea-pigs,[13] and occasionally in fox encephalitis.[19] Often the halo is poorly developed or is only transitory as in the diffuse granular inclusions of panleukopenia of cats and late stages of herpes.

It is obvious that in the definition of inclusions which has been suggested there are few morphologic criteria which one can use to determine whether an inclusion body is present in some new virus disease which may be discovered. The burden of proof falls on the investigator to demonstrate that the peculiar cellular reaction is a response which is sufficiently specific for the agent which produces it to have diagnostic value in the identification of that agent. In panleukopenia of cats the diffuse and clustered granular inclusions are specifically diagnostic; all other nuclear reactions which may produce inclusion-like structures are not.*

Summary

1. Examination of cats at intervals during the development of panleukopenia, at the height of the disease and soon afterwards revealed that the primary reactions occur in the lymph nodes and lymphoid tissue of the ileum and are soon followed by reactions in the mucosa of the intestine. The liver, pancreas, and kidney respond late in the disease and in them the resulting injury may be greater than that found in the primary centers.

2. In the lymph glands the initial response, soon after infection, is a draining off of lymphocytes from medullary spaces and cords, accompanied by severe phagocytosis of erythrocytes. The primary nodules do not change extensively during the course of the disease but the secondary centers expand slightly, develop inclusions, and later show some hyaline material.

3. The mucosa of the small intestine is damaged in the terminal portion slightly before the process extends to the duodenum. In both there is extreme destruction of villi and of the epithelium of the crypts of Lieberkühn.

4. Granular intranuclear inclusions have been found in the lymphoid

* The importance of good optical equipment, set up for maximum efficiency, is so important in studies of this sort that it seems desirable to make some mention of it. A Bausch and Lomb ribbon filament lamp, set approximately 25 inches away from the microscope, was used to give Kohler illumination. The condenser of the Zeiss microscope employed was always focused as carefully as the objective. Oil was used between the condenser and slide for critical study. Finally, the apochromatic, 90×, 1.3 n.a. objective employed was of excellent quality. It was compared with other objectives of similar specifications by the same and different manufacturers. Some objectives which were tried failed to separate clearly the individual granules of the inclusion body so that they seemed to run together in a mass, the delicate reticulum could not be clearly distinguished and often fine fibers could not be seen at all.

tissues in 3 days after inoculation but at that time are absent from the intestinal epithelium. In 6 days they are present in both and remain until the height of the disease, after which they all quickly disappear.

5. Examination of clinic animals would indicate that most of them died after the crisis had passed and that inclusions and initial reactions of lymphoid tissue, characteristic of the disease, were absent; instead, there was pronounced damage to the secondarily reacting tissues.

6. Two types of granular intranuclear inclusions were present, both in lymphoid tissue and intestinal epithelium: one has been called *clustered granular,* and the other *diffuse granular.* The developmental cycle of each has been worked out. With ageing they develop a slight basophilia superimposed on an original, strong eosinophilia and in the late stages become homogeneous in character.

7. In liver as well as in lymphadenoid and intestinal tissues homogeneous inclusions derived from plasmosomes may be found. Homogeneous inclusions derived from the granular types may be distinguished from those derived from plasmosomes by a difference in tinctorial reaction in most cases.

8. Two cells with inclusions were found which were undergoing mitosis; one was in the prophase and the other in what might be a metaphase. This is the first report of mitosis in cells bearing intranuclear inclusions of the granular type.

9. On the basis of morphology and tinctorial reactions, granular inclusions of panleukopenia of cats have been classified with those of yellow fever and burns, but differences in their developmental cycles have been pointed out. The work of Belt,[28] showing that the inclusions of burns are similar morphologically to those of yellow fever, has been confirmed.

10. By way of definition, the term, inclusion body, should be applied to those morphologic abnormalities in the cell which, when their complete cycle has been worked out, are found to have a specific or relatively specific association with the agent or pathologic condition which produces them.

We wish to express our appreciation to the Research Council of Iowa State College for the granting of funds to defray publication costs of the plates.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Drawings were outlined with a camera lucida at a projected magnification of × 3250 and have been reduced one-fourth in publication.

PLATE 78

All cells are from tissues fixed in Zenker's solution with acetic acid, except where noted, and stained with hematoxylin and eosin. Figures 1 to 30 inclusive are of cells from normal cats or those having panleukopenia.

FIG. 1. Cat 81. Epithelium, lower ileum. Hyperoxychromatism, which falls within the range of usual nuclear variation.

FIG. 2. Cat 80. Lymph nodule, ileocecal junction. Early stage in clustered granular inclusion.

FIG. 3. Cat 82. Epithelium, lower ileum. Early stage in inclusion formation.

FIG. 4. Cat 82. Epithelium, lower ileum. Coloration of inclusions characteristic of young to fully formed inclusions.

FIG. 5. Cat 81. Epithelium, lower ileum. Fully formed inclusion. Slight basophilia superimposed on original acidophilia.

FIGS. 6 and 7. Cat. 81. Epithelium, lower ileum. Late homogeneous stage of inclusion cycle. Figure 7 has a neutrophilic plasmosome to the left of the inclusion.

FIG. 8. Cat 80. From lymph node near colon. Probably early stage in formation of diffuse granular inclusion.

FIG. 9. Cat 81. Epithelium from duodenum. Young but clearly recognizable diffuse granular inclusion.

FIG. 10. Cat. 80. From lymph node near colon. Fully formed diffuse granular inclusion.

FIG. 11. Cat 80. From lymph nodule, lower ileum. Slight basophilia and condensation, evidences of ageing.

FIG. 12. Cat 81. Epithelium, duodenum. Late stage in diffuse granular inclusion. Corresponds to Figure 6 for the clustered granular inclusion.

FIG. 13. Cat. 81. Epithelium, duodenum. Nucleus shows characteristics of both diffuse and clustered inclusions.

FIG. 14. Cat 82. Epithelium, lower ileum. A neutrophilic plasmosome surrounded by a halo, chromatin partially marginated.

FIG. 15. Cat 82. Epithelium, lower ileum. A younger acidophilic and an older neutrophilic plasmosome.

FIG. 16. Cat 82. Lymph nodule, lower ileum. Neutrophilic bodies embedded in oxychromatin. Probably an atypical plasmosome.

FIG. 17. Cat 82. Lymph nodule, lower ileum. Homogeneous body intermediate in color between homogeneous stage of granular inclusion and neutrophilic plasmosome. Origin, therefore, not known.

FIG. 18. Cat 82. Epithelium, lower ileum. Prophase stage of mitosis in a nucleus which contains a clustered granular inclusion.

FIG. 19. Cat 81. Epithelium, duodenum. Late prophase or metaphase of a nucleus with an intranuclear inclusion. Mitosis probably abnormal.

FIG. 20. Cat 82. Epithelium, lower ileum. Beginning necrobiotic changes in a nucleus which has a clustered granular inclusion in middle phase of its cycle.

FIG. 21. Cat 82. Epithelium, lower ileum. Late necrobiotic changes in nucleus with older, but not yet homogeneous, granular inclusion. Ballooned, striated, cuticular border.

FIG. 22. Cat 82. Epithelium, lower ileum. Nucleus above shows karyorrhexis of chromatin. Halo gone. In color and structure resembles late herpes inclusion. Nucleus below shows chromatin in solution. Inclusion may or may not have been present in the latter.

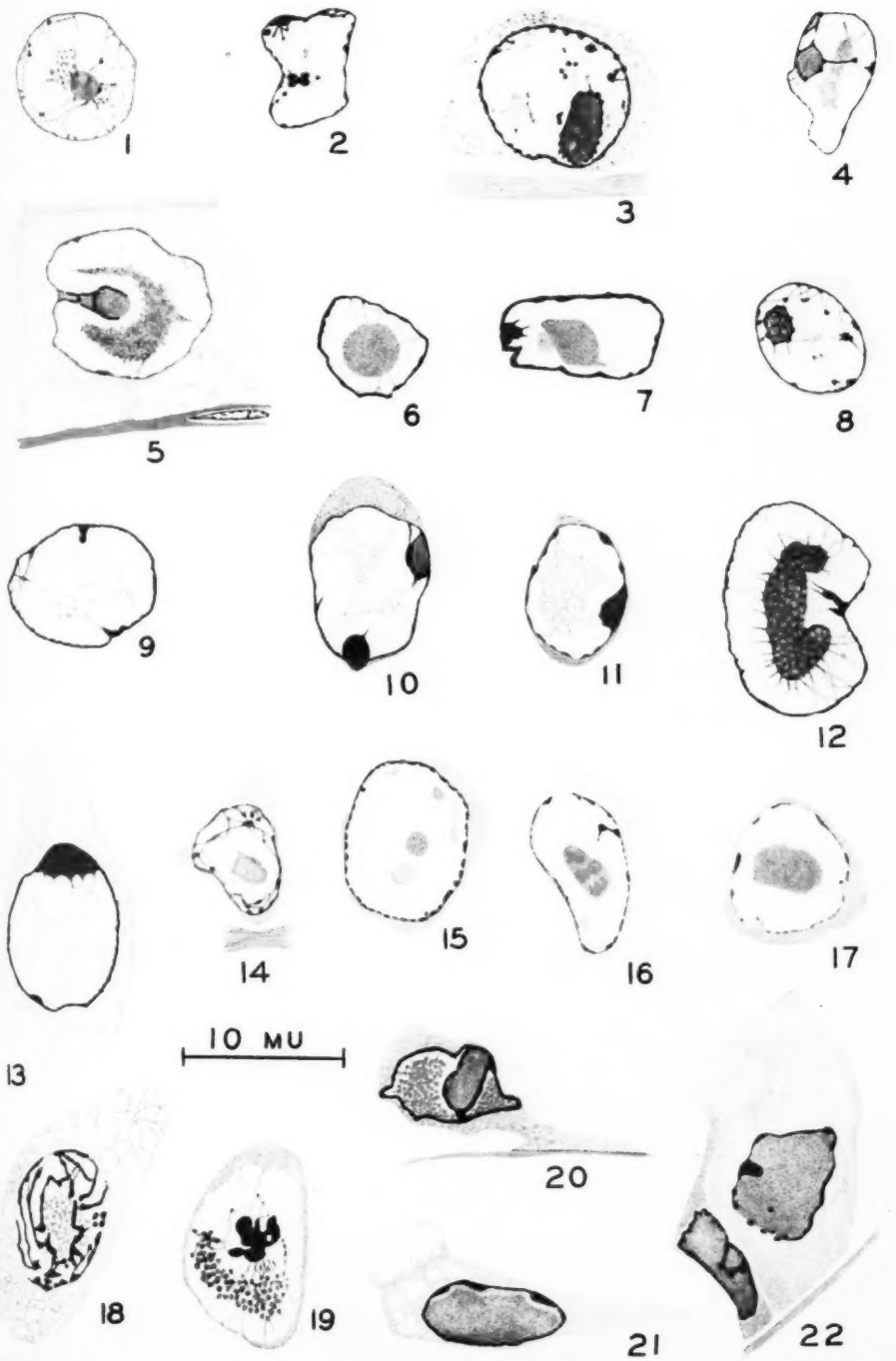
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Plate 79

Fig. 23. Cat 82. Liver cell. Typical nuclear structure. Located three cells away from one shown in Figure 25.

Fig. 24. Cat 82. Liver cell. Partial retraction of chromatin from plasmosome; cell is not preparing for mitosis.

Fig. 25. Cat 82. Liver cell. Plasmosome surrounded by halo and partially marginated chromatin. Structurally identical with intranuclear inclusions associated with some virus diseases.

Fig. 26. Cat 81. Lymph node near colon. Hypertrophy of a plasmosome with vacuolization accentuated by refractility of the margins or substance within the vacuole.

Figures 27, 28, and 29-a were drawn to show all structures of the dorsal half of the nuclear sphere.

Fig. 27. Cat 79. From lymph nodule in ileocecal junction. Early prophase in mitosis. With the formation of the spireme, the basichromatin leaves the plasmosome.

Fig. 28. Cat 79. From lymph nodule in ileocecal junction. Prophase, later than cell in Figure 27. Most of the spireme threads have left the plasmosome and gone to the nuclear margin.

Fig. 29-a. Cat 79. From lymph nodule in ileocecal junction. Prophase, later than Figure 28. All chromatin has left the plasmosome except one strand.

Fig. 29-b. Same nucleus as shown in Figure 29-a, but drawn at a fixed level of focus. Simulates an intranuclear inclusion, with halo and marginated chromatin.

Fig. 30. Cat 33. Epithelium, ileum. Empty or ghost nucleus. Margination of chromatin but no inclusion. All but a small portion of this particular nucleus was contained in a single section.

Figures 31, 32 and 33 are drawings of cells from rabbit brain showing inclusions of herpes simplex.

Fig. 31. Early nuclear reaction, margination, and oxychromatic accumulations.

Fig. 32. Oxychromatic masses and accumulating granules characteristic of the typical inclusion. Reticulum joining granules is occasionally visible.

Fig. 33. Late inclusion. Homogeneous masses and halo gone. Granules joined by reticulum fill the nucleus.

Fig. 34. Testis of rabbit inoculated with B-virus. Small blood vessel surrounded by inclusion-bearing cells. Early stage, cells at lower right and left corners; homogeneous oxychromatic masses with granules. Middle stage, upper cell; medium halo, granules on conspicuous reticulum. Later stage, middle right; halo almost gone; granules more numerous and closer but reticulum still clear.

Figures 35 to 40 are drawings of liver cells from human cases of severe burns. Formalin fixation. Case numbers are from the pathology records of the University of Toronto. A-39-32: F., 51 years. Burn from wax, died 3 days later. A-157-34: M., 37 years. Burn from gasoline, died 3 days later. A-306-34: M., 55 years. Burn from steam, died 2 days later.

Fig. 35. A-306-34. Most of the chromatin has marginated. Some lagging of the amphinucleolus. Scattered clusters of acidophilic granules.

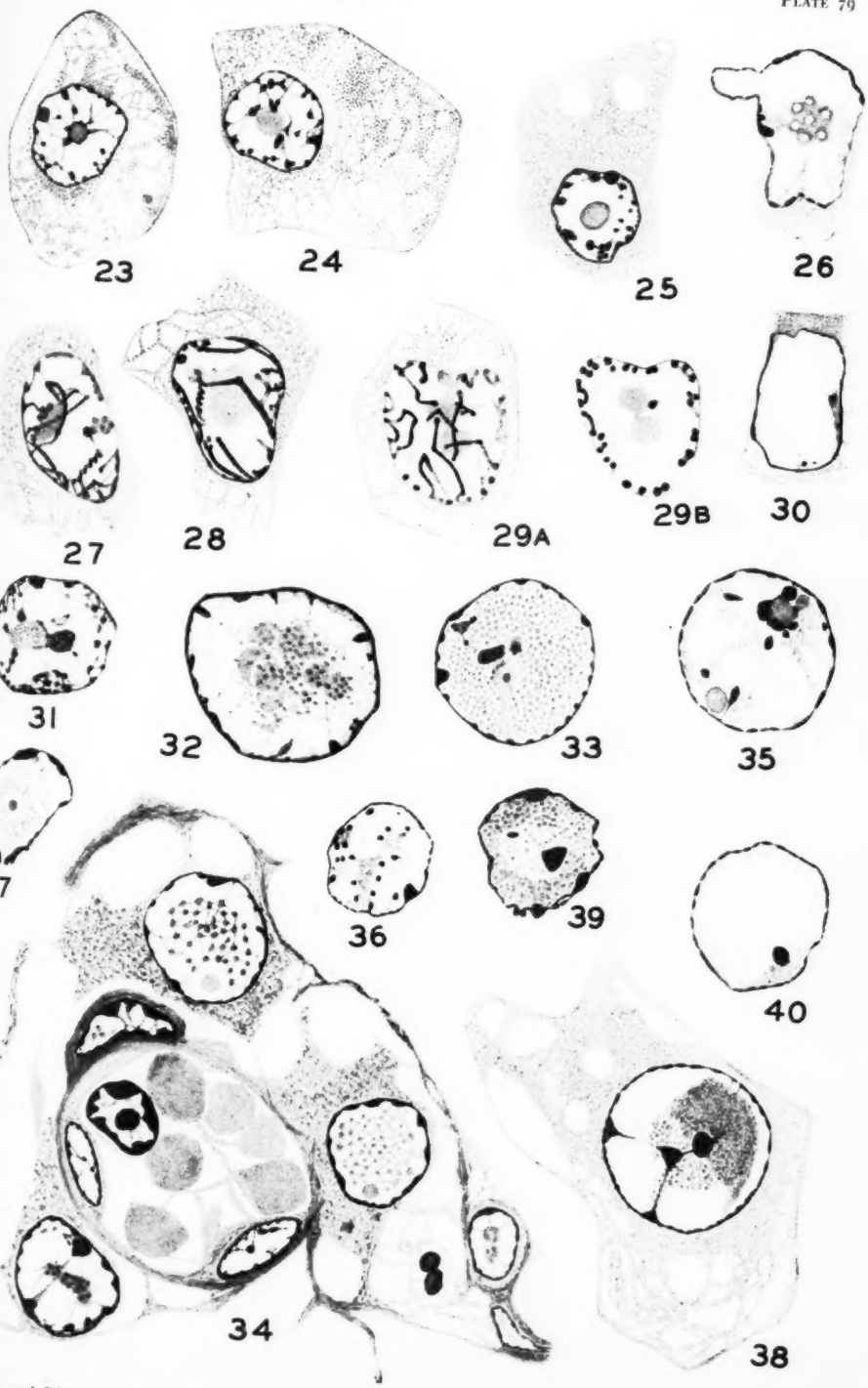
Fig. 36. A-157-34. A few basichromatin granules associated with acidophilic clusters.

Fig. 37. A-39-32. Early karyorrhexis of marginated basichromatin. Oxychromatin clustered in center to form inclusion body. Similar to yellow fever inclusion.

Fig. 38. A-306-34. Resembles closely a fully formed yellow fever inclusion. Granules slightly less refractile in material available for comparison.

Fig. 39. A-39-32. Nuclear death superimposed on inclusion. In addition to shrinkage and beginning karyorrhexis, there is liquefaction of basichromatin, which causes inclusion to resemble that of herpes.

Fig. 40. A-306-34. Empty or ghost nucleus, often found in this material and in yellow fever.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SARCOSPORIDIOSIS OR TOXOPLASMOSIS IN MAN AND GUINEA-PIG *

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INTRODUCTION

In many of the texts on human parasitology is a chapter entitled "Parasites of Undetermined Nature," and according to Craig and Faust [1] "Those belonging to the Sarcosporidia and to Toxoplasma are the most important . . ."

The exact taxonomic status of the Sarcosporidia has not been determined. The order Sarcosporidia, Bütschlii, 1882, containing only one genus, Sarcocystis, Lankester, 1882, has been considered closely allied to the Cnidosporidia [2] or Neosporidia,[3] but Wenyon [4] disapproved of this classification. Belding [5] included the order Sarcosporidia in the subclass Acnidosporidia (characterized by the production of simple spores during the life of the sporozoon), class Sporozoa. Approximately 50 species of Sarcocystis have been recorded in mammals, including man, rat, mouse, sheep, cattle, pig, horse, rabbit, dog, cat, deer; birds; reptiles; and recently in fish.[6] It is probable that there are fewer species, although Alexeieff's [7] suggestion that only one species exists may be too radical.

The epidemiology and life cycle of Sarcosporidia are incompletely known. The evidence suggests that, following the consumption of infected food, sporozoites are liberated from the adult cysts, penetrate the intestinal epithelium, reach the lymphatics or possibly the blood stream, and eventually localize in the skeletal muscles. Animals on the whole are tolerant to the presence of sarcocysts, although various debilities have been associated with the infection (see Scott [8] for review). Pfeiffer [9] first demonstrated that emulsions of the parasite of sheep, *Sarcocystis tenella,* when injected subcutaneously, can kill mice and guinea-pigs. The name of "sarcocystin" was applied to this toxic substance by Laveran and Mesnil.[10]

In his authoritative monograph on the Sarcosporidia, Babudieri [11] accepted the following as authentic reports of human sarcosporidiosis:

Baraban and St. Remy [12]	1894	Manifold [16]	1924
Vuillemin [13]	1902	Lambert [17]	1927
Darling [14]	1909	Vasudevan [18]	1927
Darling [15]	1920	Bonne and Soewandi [19]	1929
To these may be added:			
Feng [20]	1932	Hewitt [22]	1933
Price [21]	1933	Gilmore, Kean and Posey [23]	1942

* Received for publication, June 5, 1944.

We are in agreement with Babudieri [11] in regard to these cases which he regarded as authentic (within the limitations of the discussion in this paper) and also with his rejection of many reported cases because he considered them incorrect, doubtful, or lacking in sufficient data for review. The latter group includes the cases of Lindemann, Leuckart, Koch and Gaffky, Rosenberg, Kartulis, and others. (See Babudieri for review.) The case of Kartulis [24] has in the past been rejected by some authorities and credited by others. We are inclined to believe that this is an authentic case, but proof at this date seems impossible.

The case of "bone sarcosporidiosis" reported by Cone [25] must be questioned. In 1928, Naidu [26] published a record of a patient who probably was the same one described by Vasudevan [18] in 1927. The infection observed by Hertig [27] in the myocardium of an infant has been redescribed by Pinkerton and Weinman [28] as toxoplasmosis. Price [21] noted cases of "Scoglia" (probably Scaglia [29]) and of Askanazy,[30] and Rivas [31] mentioned those of Nancy and A. J. Smith, but we have been unable to find records of these cases.

Morphology of the Parasites Hitherto Reported as Sarcosporidia in Man

Although the morphology of the Sarcosporidia varies considerably depending upon the species(?), host, and age, the following description by Craig and Faust [1] of the human parasite, *Sarcocystis lindemanni*, Rivolta, 1878, is generally applicable:

"When fully developed, *S. lindemanni* consists of a cylindrical, elongated or fusiform body, hyaline in appearance, with more or less pointed ends, lying in the affected muscle fibers. It is enclosed in a membrane and contains myriads of round and crescent-shaped spores. In 1843, Miescher found these bodies in the muscle fibers of mice; since that time they have been known as 'Miescher's tubes.' They vary much in size, from forms measuring as much as 5 centimeters in length to others so small that it requires a microscope to detect them. When visible to the naked eye they appear as minute white streaks within the muscle fibers. When removed from a muscle fiber each parasite consists of a cylindrical whitish tube with somewhat pointed ends and a finely lobulated surface. The membrane forming the outer coat of the organism may show a radial striation and from this membrane firm prolongations extend, dividing the tube into separate compartments, of which the outer contain round cells, while in the inner, fully developed compartments there occur the characteristic crescentic bodies called spores. Rounded, ovoidal, elongated or sickle-shaped spores are produced which are known as 'Rainey's corpuscles.' The spores measure from 12 to 16 microns in length and from 4 to 9 microns in breadth; when properly stained they are seen to contain an elongated nucleus situated near the more rounded end of the spore. This nucleus consists of a nuclear membrane and a central karyosome. Many thousands of spores are contained in a fully developed parasite."

In 1894 Baraban and St. Remy [12] described the first case of human sarcosporidiosis which has been universally accepted as authentic. The

parasites were found in the laryngeal muscles of a man executed by hanging. The parasites were large, measuring 1.6 mm. by 0.17 mm., and distended the muscle fibers. The capsules were thin, but striated. Room compartments contained individual sporozoites measuring 8 to 9 μ in length. No inflammatory reaction was present.

Darling,[14] in 1909, recorded what he considered the third instance of human sarcosporidiosis. He was unaware, apparently, of Vuillemin's [13] report but accepted at that time the case of Kartulis.[24] The parasites were found in the biceps of a Negro ill of typhoid fever. Darling's observations may be recorded in detail:

"Here and there were oval or round dotted bodies about the width of a striated muscle fiber, their length being about twice that. The muscle fiber was not distended by the presence of the dotted body. One oval body measured 0.084 mm. in length and 0.027 mm. in width. One of the bodies in cross section was circular and was 0.021 mm. in diameter. The oval or round bodies were strongly contrasted from the eosin-staining muscle fiber by the very pronounced blue stippling of the former. This stippling was seen under the high power to be due to the nuclei of small oval sporozoites. In this section there was some hyaline change in the muscle fibers, both in the infected and the non-infected fibers. Occasionally within the capillaries near one of the oval bodies there was a slight increase in the number of polymorphonuclear leucocytes and there were a few foci of acute myositis involving a single fiber. Some of the specimens showed a cross-section of the sporozoön, in which it was seen that the latter was just within the sarcolemma. No matter how wrinkled or distorted the muscle fiber might be, the sporozoön, with its very thin refractile membrane, was seen to preserve its circular or oval outline. Under the highest powers the sporozoön was seen to be made up of hundreds of little oval vesicular bodies having a round nucleus at one end. The sporozoite took the eosin irregularly and appeared to be vacuolated. The little sporozoites were decidedly vesicular and were either round or oval. All had one nucleus and very rarely two nuclei placed at opposite ends of the short axis of the sporozoite. The sporozoites were closely packed within the mother capsule or membrane, yet without any arrangement suggesting a room system. The measurement of sporozoites in the section was: length 4.25 microns, width 1.75 microns."

It was obvious to Darling [14] that the parasite he observed differed in several respects from that described by Baraban and St. Remy [12] and also from animal Sarcosporidia such as *Sarcocystis tenella* (in sheep) (Fig. 4) and *Sarcocystis miescheriana* (in pigs) (Fig. 5). Darling believed that the parasites in his human case differed from those in animals because man was a strange host and hence the parasite was unable to complete its life cycle.

The experiments of Darling [32] presented what has been considered proof of his theory. He fed 6 guinea-pigs rat muscle naturally infected with *Sarcocystis muris,* and found that 2 pigs killed 6 months later had parasites in the skeletal muscles similar to those seen in his human case. These uncontrolled experiments were supposed to have confirmed the work of Negri,[33] who fed 11 guinea-pigs infected rat muscle and found parasites in 9. No parasites were found in 12 control pigs. Negri was

impressed by the differences between the parasites in his experimental animals and in rats, and attributed the morphologic change to the fact that the parasites were in a strange host, a theory popularized by Darling. Both Negri and Darling used the guinea-pig as their experimental animal because they believed that this animal was not naturally infected with Sarcosporidia.

Our review of the reported cases of human sarcosporidiosis has revealed that they may be divided into two groups: Group I in which the parasites, cyst and individuals, are large, with well defined, striated cyst walls and inner room compartments. This parasite resembles the Sarcosporidia of animals such as sheep and pig. In this category belong the cases of Baraban and St. Remy,[12] Vuillemin,[13] Bonne and Soewandi,[19] Feng,[20] Price,[21] and Hewitt.[22]

Group II is characterized by the smaller size of the cyst and its individual members, a delicate, ill defined, nonstriated capsule and the absence of internal septa. Here may be placed the two cases of Darling [14, 15] and those observed by Manifold,[16] Lambert,[17] Gilmore, Kean, and Posey.[23]

Until a few years ago the following case would surely have been listed as another instance of sarcosporidiosis and, according to the foregoing classification, would belong to group II.

Report of Case

Patient A. W. (Gorgas Hospital chart no. 530409, Board of Health Laboratory autopsy no. 14230) was a Negro housewife, 48 years old, who died in Gorgas Hospital, Ancon, Canal Zone, on August 17, 1943, 4 hours after admission for acute dyspnea and retrosternal pain.

Background. The patient was born in Jamaica, British West Indies, and lived there until the age of 25. The family history was not contributory. At the age of 25 she went to Bocas del Toro, Republic of Panama, where she worked as a seamstress for 12 years, then spent some years in Almirante and Puerto Armuelles, Republic of Panama, and came to the city of Panama about a year before her death. Her health had generally been good. She had had a child which died in infancy before she left Jamaica. She married in Bocas del Toro at about the age of 28 years and had two abortions in the early months of pregnancy.

A year before her death she was admitted to The United Fruit Company Hospital in Puerto Armuelles, Republic of Panama, where a colpotomy for pelvic abscess was performed. Convalescence was uncomplicated. No history of antiluetic therapy was obtained. Her food habits were not remarkable and were typical of Jamaicans. She ate no raw meat. Her only obvious contact with animals was with a dog, a household pet not available for examination. It was known that the house in which she resided in Panama during the last year of her life was infested with rats, and contamination of food was possible. Her husband was living and well, and declined to permit a muscle biopsy.

History of Terminal Illness. Three weeks before admission the patient became ill, complaining of epigastric pain, exertional dyspnea, orthopnea, and swelling of the right leg. She continued her household duties for a fortnight, however, but then took to bed. On the day of admission, sharp, severe, substernal pain radiating to

the back, marked dyspnea, abdominal distention, profuse diaphoresis, and questionable fever brought her to the hospital.

Physical Examination. The patient was a somewhat obese Negro woman in acute distress. Temperature was 97.0° F.; pulse rate, 120 per minute; respiration, 32 per minute; blood pressure, 120 mm. Hg systolic and 90 mm. Hg diastolic. A systolic murmur was heard at the apex of the heart, but no other abnormalities were noted upon physical examination of the heart and lungs. No peripheral edema was present.

Course in Hospital. One hour after admission the retrosternal pain became even more severe and she vomited. She was given morphine sulphate, grains 1/6, without relief. Two hours after admission she appeared to be in extreme shock. Her skin was cold and clammy, the blood pressure could not be obtained, the heart sounds were poor in quality and the cardiac rhythm became totally irregular. She died 4 hours after admission without rallying. No laboratory procedures were conducted. The clinical diagnosis was coronary occlusion.

AUTOPSY FINDINGS

A complete autopsy, including examination of the head, was performed 11 hours after death. The anatomic diagnoses included: parasitization of heart (sarcosporidiosis or toxoplasmosis); cardiac hypertrophy (360 gm.); chronic interstitial myocarditis, slight; area of focal encephalomalacia of brain, small; chronic diffuse thyroiditis (Hashimoto type); hemorrhages of thymus, small, focal, terminal; acute and chronic passive congestion of liver; fibrous perisplenitis, old; fibromyomata of uterus; chronic pelvic peritonitis; chronic salpingitis, bilateral; atrophy of ovaries; obesity, moderate; general arteriosclerosis, slight; post-mortem degeneration of pancreas and kidneys, moderate. The exact cause of death was not determined nor could the pathologic findings be correlated satisfactorily with the clinical course.

Since the parasites were observed only within the heart, description will be limited to that organ. No skeletal muscle was taken for examination. No parasites were found in 167 sections of the brain.

Gross Examination of the Heart

No excess pericardial fluid was present. The heart was slightly enlarged, weighing 360 gm. after removal of blood clot from the chambers. The apex was located in the fifth interspace, 11.0 cm. to the left of the midsternal line. The epicardial surface was smooth and free from adhesions to the pericardium. A moderate amount of subepicardial fat was present. The myocardium was uniformly firm and reddish brown. No areas of myocardial necrosis or fibrosis were recognized. The endocardium was smooth and glistening. The valve leaflets were delicate and appeared competent. No lesions of the aortic valves suggestive of syphilis were present. Coronary sclerosis was slight; no occlusion was observed.

Microscopic Examination of the Heart

Thirteen blocks were taken from the interventricular septum, the left ventricular wall, and the right ventricular wall. Sections of the auricular walls were not taken. From these thirteen blocks, 365 sections were prepared. In six of the blocks, including all taken from the right ventricular wall, no significant abnormalities were noted. The subepicardial fat was free of inflammatory reaction. The myocardial fibers tended to be large and contained a small to moderate amount of yellowish perinuclear pigment. A few small patches of myocardial fibrosis were found. The endocardium was not thickened and no verrucae were seen.

In seven sections of the left ventricular wall there were found nine parasitic cysts. Since all of these cysts were similar in character, it may be sufficient to describe a typical one (Fig. 1).

Within a single swollen muscle fiber, cut in cross or slightly tangential section, was a cyst measuring 60 by 50 μ. The cyst was packed with numerous elongated, slightly elliptical bodies which, for purposes of convenience in description, were called sporozoites. These bodies averaged 5 μ in their greatest dimension and had nuclei approximately 1 μ in diameter generally located at one pole. The cyst possessed a delicate capsule, or membrane, which stained poorly in routine hematoxylin and eosin sections, but in van Gieson preparations stood out as a shiny, refractile structure. It could not be ascertained whether the capsule was of parasitic or muscle-fiber origin. No striation of the capsule and no internal septa could be demonstrated. Toward the periphery of the cyst, just beneath the capsule, elongated sporozoites were not seen. Instead, there was a row of tiny nuclei representing, possibly, immature sporozoites.

The following were measurements of other cysts: 50 by 27 μ; 105 by 47 μ; 80 by 52 μ; 35 by 26 μ. Attempts to section, in serial fashion, some of the parasites were productive of the following results. In most instances serial sections could not be obtained, for the parasites were found in only one slide. In one instance, 15 consecutive sections, cut at intervals of 5 μ, included the same cyst. It was estimated, therefore, that the size of this cyst was at least 75 by 35 by 25 μ. In another instance it was possible to obtain 9 consecutive sections, each containing the same cyst, and from the method of preparation of this material and from a study of the sections it appeared likely that the entire parasitic structure was included in the material studied. The dimensions of this cyst were 105 by 47 μ.

A striking feature of the sections was the virtual absence of inflam-

matory reaction in the myocardium adjacent to the parasites. In fact, in only one section of the left ventricular wall could an area be found in which there was a distinct chronic inflammatory reaction with infiltration by lymphocytes. Scattered within other sections, however, there could be found a slight inflammatory reaction which bore no direct relation to the presence of the cysts. No spirochetes were found in sections stained by Levaditi's method.

Several of the cysts were ruptured by microdissection of stained material and the individual parasites studied. These averaged 5 by 1 μ and were characterized by an elongated, elliptical or crescentic shape, and by proportionately large nuclei which, for the most part, were located at one pole and produced a bulge in the outline of the sporozoite. The illustrations published by Darling [32] of the sporozoites seen in his "experimental guinea-pig sarcosporidiosis" are accurate representations of the individual sporozoites in this human material.

Parasites in Guinea-Pig

In 1942 Gilmore, Kean, and Posey [23] reported the presence of parasites in the heart of a Panamanian girl, 12 years old, which morphologically appear identical with those in the current case. At that time, following suggestions by Augustine [34] and Weinman,[35] serious consideration was given to the possibility that the parasite was Toxoplasma. The parasite was classified as Sarcosporidia, however, for various reasons which are recorded in the report; among those reasons was the fact that the parasite was indistinguishable from those in Darling's [15, 32] illustrations and those of subsequent cases,[16, 17] the diagnosis of which had not been questioned.

When the current case appeared we determined to investigate the muscles of guinea-pigs, for if Darling were wrong in his classification, then all cases in group II (page 470) were in an incorrect category. It did not seem reasonable that Sarcosporidia, which is so widespread in its distribution, should be absent in the guinea-pig.

Between September 10, 1943, and November 13, 1943, the skeletal muscles of 60 laboratory guinea-pigs, *Cavia cobaya*, were examined. These pigs were imported from the United States where they had been purchased from a commercial dealer; presumably they had not been used previously for experimental purposes. They were kept under the usual laboratory conditions until sacrificed for complement. No muscle parasites were recognized grossly. Blocks of thigh and abdominal muscles of all were fixed in formol-alcohol, and prepared by the usual paraffin method, with hematoxylin and eosin staining.

In 5 of the 60 animals parasites resembling those described and illustrated by Negri [33] and Darling [14] were found in the skeletal muscles. Complete autopsies were done on 2 of these 5, the bodies of which had been saved in the ice box. In 1 of these 2 guinea-pigs parasites were found only in the thigh muscles. Examination of the masseter, pectoral, abdominal, and tongue muscles, and of brain, heart, lungs, liver, spleen, and kidneys was negative. In the other pig parasites were found in thigh and paravertebral muscles, brain, and kidney; no parasites were noted in sections of heart, lungs, liver, spleen, tongue, esophagus, and trachea.

MUSCLE

The parasites were not significantly different in any of the 5 pigs in which they were found. About half of the muscle sections of these pigs had cysts. The parasites were more numerous in sections of the thigh than in the other muscle groups such as abdominal, pectoral, and masseter. The greatest number found in any one section was three well formed cysts (Fig. 2). The cysts were located within individual muscle fibers, generally in an eccentric position. In a few instances the cysts seemed to be located between muscle fibers.

Cross and tangential sections of cysts varied in size from 20 by 26 μ to 48 by 18 μ. Since none of the cysts was cut longitudinally, their length was not determined. In one instance it was possible to obtain six serial sections cut at intervals of 5 μ, which would make the cyst at least 30 μ long. Most of the cysts were cut in cross section and appeared as circular nests; others were cut obliquely and were ovoid in shape. The parasitized muscle fibers were swollen and a few showed hyaline degeneration. Each cyst possessed a thin, delicate capsule or limiting membrane measuring approximately 1 μ in thickness. This membrane could be seen best in sections stained with van Gieson's picro-acid fuchsin by which it was stained red and stood out in marked contrast to the greenish yellow fiber. Sarcolemma, however, possessed the same tinctorial properties. We could not determine whether the capsule was of parasitic or muscle origin.

Each cyst was composed of, or filled with, many tiny bodies which, for purposes of convenience, were termed sporozoites. Under the low power objective the sporozoites were noted as fine basophilic stippling within the cyst. Under higher magnification the sporozoites were seen to be elongated. They were so closely packed that counting was difficult but it was estimated that each cyst contained about 150 sporozoites. In some of the cysts the bodies were disposed in a haphazard fashion, whereas in others they tended to collect in groups. Toward the periphery of the cysts, the elongated bodies were not present. Instead,

tiny spherical or ovoid basophilic bodies resembling nuclei of the sporozoites were noted.

In order to study the individual sporozoites, the following technic was employed. The coverslip was removed from a stained section and a drop of balsam was placed on the tissue. Practically all of the tissue surrounding a cyst was dissected away and the débris was flushed off with xylol. A tiny drop of balsam was placed directly upon the cyst and the coverslip returned. Pressure on the coverslip was applied. This was sufficient to rupture the cyst capsule and the extruded individual sporozoites could then be examined under the oil immersion objective and measured. The dangers of regarding the measurements of sporozoites treated in such a fashion as *in vivo* dimensions must be obvious.

In these preparations the sporozoites measured 5 by 1 μ. Some were crescentic or banana-shaped. Most of them tapered at both ends, but several were bluntly rounded at one pole. A dark-staining nucleus was present near the tip of each parasite, and in many it was found to occupy the extreme distal part of the sporozoite. In these sporozoites the nucleus was found to accommodate itself to the taper of the corpuscle and appeared as a dark-staining, roughly triangular body with a somewhat rounded base. The nuclei in other sporozoites were found to be nearer the center, but it was difficult to find any with a centrally placed nucleus. A section stained by Heidenhain's iron hematoxylin technic showed the nuclear chromatin to be arranged chiefly around the periphery of the nucleus. No definite intranuclear structures were ascertainable. The cytoplasm of some of the corpuscles was distinctly granular, and vacuoles were present in a few of them. The morphologic characteristics of the cysts and of sporozoites appeared identical with those so beautifully illustrated by Darling.[14, 32]

BRAIN

Parasites were found in the brain of 1 of the 2 guinea-pigs upon which complete autopsies were done. The cysts (Fig. 3) were ovoid or circular in outline and measured from 20 to 25 μ in diameter. No outer membrane or capsule could be recognized and the cysts seemed to be limited only by the surrounding parenchyma. As many as 80 sporozoites could be counted in some collections; these resembled those found in the muscles, but appeared scattered in an ill defined, faintly basophilic ground substance. There was no surrounding cellular reaction, but the leptomeninges showed a slight chronic inflammatory exudate. These cysts resembled the illustrations of toxoplasmosis in guinea-pigs (Markham [36]), wild rats (Perrin, Brigham, and Pickens [37]), and mice (Weinman [38]).

KIDNEY

Within the lumen of a renal tubule was a circular nest of parasites measuring 35 μ in diameter. This nest was composed of 50 or 60 nucleated bodies scattered in faintly basophilic stroma. Nearby was a collection of 7 smaller cysts ranging from 15 to 20 μ in diameter. Each of these contained 10 to 15 nuclear structures scattered in a similar stroma. These aggregates resembled those found in the brain and muscles, but there was less differentiation of the internal structure. Post-mortem degeneration was considerable and may have played a rôle in obscuring the inner structure of these cysts. There was no surrounding inflammatory reaction.

COMMENT

In the absence of inoculation and serologic studies, absolute identification of the parasites in the current human case and in the guinea-pigs is impossible. On morphologic grounds, however, the following statements appear warranted:

1. The parasites in our human case are similar to those described by Darling and others (group II) as Sarcosporidia.

2. The classification of these parasites as Sarcosporidia has been based mainly upon Darling's experiment. We found parasites occurring spontaneously in guinea-pigs similar to those which Darling thought he had transmitted experimentally. Negri's work [33] requires confirmation.

3. The parasites in both group II and in the guinea-pigs resemble Toxoplasma more than Sarcosporidia. Toxoplasmosis has been reported in guinea-pigs by Mooser,[39] Markham,[36] and Sabin and Olitsky,[40] but parasites in peripheral skeletal muscle were not mentioned. A detailed discussion of Toxoplasma in man need not be presented for several articles have summarized current knowledge (Pinkerton and Weinman,[28] Wolf, Cowen, and Paige,[41] etc.). The cysts, both in group II and in guinea-pigs, are somewhat larger than those generally described in chronic toxoplasmosis,[37,38] possibly because they are present in the more abundant cytoplasm of striated muscle.

4. Although the parasites in group II of the human cases and in the guinea-pig appear similar, it has not been established that they are identical. In fact, there is no proof that the parasites in the muscles, brain, and kidney of the guinea-pig are the same.

It is of some interest that three instances of this type of human infection should have been found in one laboratory when so few cases have been recorded all over the world. Darling [14] reported in 1909 the parasites in the biceps muscle of a patient ill of typhoid fever. The

second case (Gilmore, Kean, and Posey [23]) was found in 1941 while searching for *Trypanosoma cruzi* in the heart of a child. In the current case many blocks of heart muscle were taken because Chagas' disease was suspected at the autopsy table but the parasites were first seen by a technician (Mr. J. M. Benevides) who picked up a slide to check its stain. (Darling's second case [15] was from the Federated Malay States.) *

Summary

1. The literature on human sarcosporidiosis was reviewed and the reported cases were divided into two groups:

Group I, in which the parasites were characterized by the large size of the cysts and sporozoites, striated capsules, and internal septa. This group resembled animal Sarcosporidia such as *Sarcocystis tenella* (sheep) and *Sarcocystis miescheriana* (pig). These were regarded as authentic cases of human sarcosporidiosis.

Group II, in which the parasites were characterized by smaller size of the cyst and sporozoites, and absence of striated capsule and internal septa. These cysts resembled parasites which Darling thought he had transmitted to guinea-pigs by feeding them rat muscle infected with *Sarcocystis muris*.

2. A case is reported in which parasites in the heart of a Negro woman belonged in group II.

3. The spontaneous occurrence in the skeletal muscle of 5 of 60 guinea-pigs of parasites morphologically indistinguishable from those which Darling claimed to have transmitted experimentally was observed. The presence of parasites resembling Toxoplasma in the brain and kidney of one of these pigs suggested strongly that the muscle parasites were also Toxoplasma.

4. Since the classification of group II as Sarcosporidia stemmed from Darling's questionable experiment, it was considered probable that the parasites in group II were Toxoplasma rather than Sarcosporidia.

5. In the absence of serologic and inoculation experiments, final classification of the parasites was not attempted. The possibility that the parasites in group II and in the muscle of guinea-pigs were neither Sarcosporidia nor Toxoplasma was not excluded.

We are indebted to Dr. Carl M. Johnson, Gorgas Memorial Laboratory, Panama, R. of P., for the photomicrographs.

* Since this paper was submitted for publication, parasites resembling Toxoplasma were found in sections of the brain and heart of a child upon whom an autopsy was performed in this laboratory in 1936. (Tomlinson, W. J. Human chronic toxoplasmosis. *Am. J. Clin. Path.* In press.)

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[*Illustrations follow*]

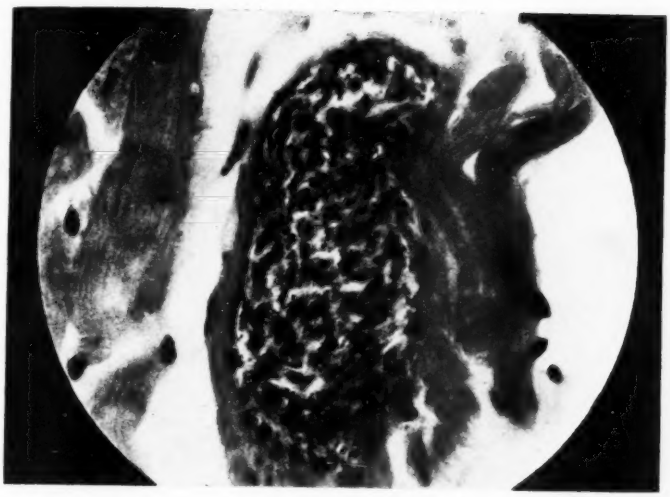
DESCRIPTION OF PLATES

PLATE 80

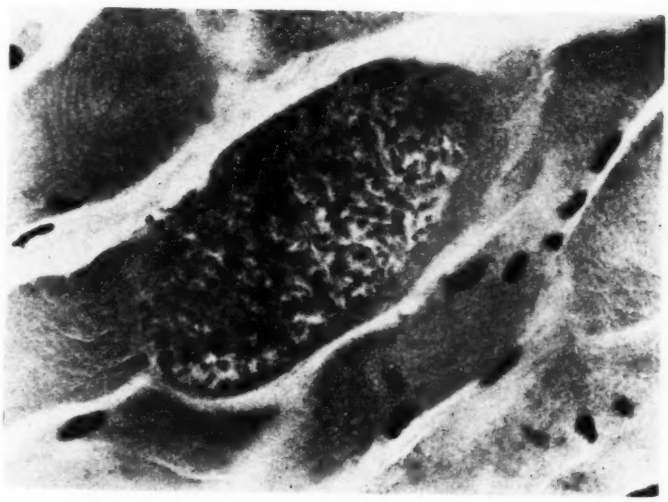
FIG. 1. Cyst in human cardiac muscle. (Patient A. W.) × 850.

FIG. 2. Cyst in thigh muscle of guinea-pig. Resemblance to human parasite in Figure 1 may be noted. × 850.

1



2



Kean and Groscott

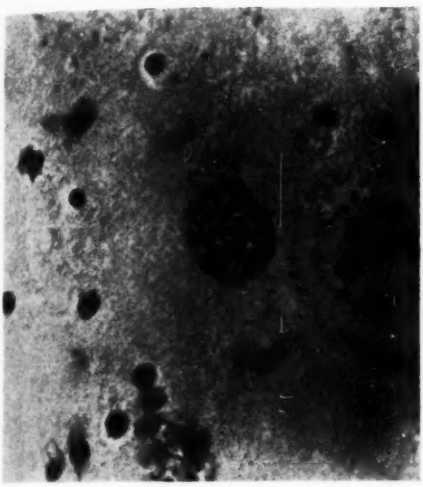
Sarcosporidiosis or Toxoplasmosis

PLATE 81

FIG. 3. Cyst or pseudocyst resembling Toxoplasma in brain of guinea-pig. A second cyst (out of focus) is present at the lower border of the field. × 850.

FIG. 4. *Sarcocystis tenella*. Parasite in heart of sheep, showing large cyst and individual sporozoites. Internal septa and a well defined capsule can be seen. × 850.

FIG. 5. *Sarcocystis miescheriana*. The cyst in cardiac muscle of a pig (not guinea-pig) has a thick striated capsule. × 850.



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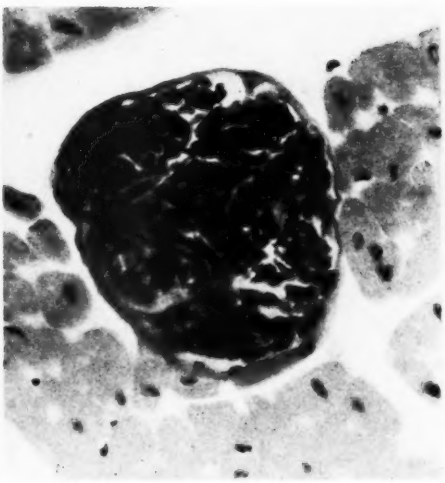

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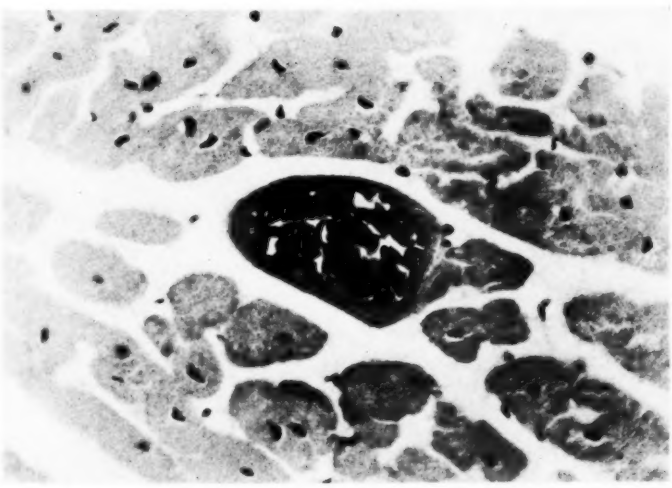

5

HEALED OR ARRESTED PULMONARY COCCIDIOIDOMYCOSIS

Correlation of Coccidioidin Skin Tests with Autopsy Findings *

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The renaissance of our knowledge of infections due to *Coccidioides immitis* occurred in 1937 with the description by Dickson,[1] Dickson and Gifford,[2] and Gifford, Buss, and Douds [3] of a widespread, benign, pulmonary infection in the San Joaquin Valley of California. Prior to that time the general concept of coccidioidal granuloma as a disseminated granulomatous disease with a 50 per cent mortality held sway. The demonstration that the minor respiratory illness often associated with erythema nodosum and commonly called "Valley Fever" was due to *C. immitis* stimulated widespread epidemiological study. Added clinical interest was aroused by the variety of pulmonary manifestations reported in early coccidioidomycosis, to wit: Bronchial pneumonia, subacute and chronic cavitation resembling tuberculosis, and calcified pulmonary lesions. The relationship of healed and calcified pulmonary lesions to positive coccidioidin skin tests forms the basis of this report.

Skin testing of patients for sensitivity to coccidioidin as a diagnostic measure or in epidemiological surveys has demonstrated a surprisingly high incidence of positive cutaneous tests depending largely on the locality of the study. The main contributors to our knowledge of this work have been Gifford, Buss, and Douds,[3] and Smith [4] in California. Farness [5] collected a series of such tests which ranged from 90 per cent positives in school children of the Pima Indian Reservation of Arizona and 58.2 per cent positives in school children of Kern County, California, to no positive reactions among school children of Philadelphia, New York, and Ann Arbor. A small series of adults in these eastern centers showed 1 to 5.9 per cent positives. The recent interesting experiences of the Medical Corps of the Army in California and Arizona have substantiated and added to the work of the earlier investigators.[6, 7]

The observations of Aronson and Gallagher [8] on Pima Indian children revealed such a high incidence of positive cutaneous reactions that the material was first thought to be nonspecific. However, the same antigen when used in testing Philadelphia children gave no positive reactions. Of greater interest was the fact that roentgen studies of the

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Indian youngsters showed calcified hilar lymph nodes, usually associated with tuberculous infections. Of extreme interest to epidemiologists is the isolation of *Haplosporangium parvum* from rodents in this region by Emmons.[9] This organism, according to the author, is "genetically related to *C. immitis* and in some manner to coccidioidomycosis."

Quiescent and calcified pulmonary lesions of coccidioidomycosis have been described on a number of occasions. A report of such lesions and a summary of the literature are found in a paper by Cox and Smith.[10]

The clinical material at the Santa Fe Coast Lines Hospital in Los Angeles is particularly suited for a study of the type undertaken. Patients admitted here are largely employees and their families. As such they come to the hospital from Arizona, New Mexico, and California. Northern California furnishes no patients, but the San Joaquin Valley, the Bay region about San Francisco, and all of Southern California provide a great number. As members of the hospital association, these patients return year after year for follow-up or for care of new illnesses. Many are observed over a period of 25 or 30 years. By routine testing of all hospital patients, we expected eventually to see in their final illness and to examine at autopsy a certain number of those upon whom skin tests had been done.

Methods

The coccidioidin used for the skin tests was obtained from C. E. Smith of Stanford University School of Medicine who has prepared the antigen for a number of epidemiological surveys. The method of its preparation is given in the paper by Aronson and Gallagher.[8]

For routine skin tests, 0.1 cc. of a 1:100 dilution of coccidioidin was used. The tests were read, together with the controls, at 24 and 48 hours. An area of erythema of 1 cm. or more was accepted as a positive reaction. For the tuberculin skin tests a purified protein derivative was employed.

Antigens listed in Tables II and III were prepared in the same manner as coccidioidin.

The lungs of autopsied cases were removed intact for roentgen ray studies. In this way calcified and fibrotic areas were localized and then excised from the lungs and peribronchial lymph nodes. One-half of the excised material was finely divided by grinding in a mortar with alundum. The remainder was decalcified and sectioned. The finely divided material, after standing in 0.05 per cent copper sulphate solution about 18 hours, was injected into the testicles of guinea-pigs. In some in-

stances it was impossible to follow this procedure as the lungs had been fixed in formalin prior to examination.

Results

Coccidioidin skin tests were performed on a total of 1,165 patients. Of these, 302, or 25.9 per cent, were positive reactors. Table I shows the geographical distribution and the results of the skin tests. It is evident that there are three major areas which are sources of infection in our material. The incidence of infection in these areas was as follows: The San Joaquin Valley, 62.8 per cent; Arizona, 28 per cent; and Texas, 35.7 per cent.

Attention is directed to the rather high percentage of positive reactors from the San Francisco Bay area. This figure is quite out of proportion to the known geographical distribution of acute coccidioidomycosis and is probably due to infections contracted in the San Joaquin Valley while on runs between San Francisco and the southern end of the Valley. The low incidence of coccidioidin positivity in Southern California is indeed quite surprising. However, the Santa Fe Railroad has not had a direct line between Los Angeles and San Francisco through the San Joaquin Valley until recent years. Moreover, we have known of only one case of coccidioidomycosis occurring in the vicinity of Los Angeles until the publication of the recent experiences of the Army. Except for these two discrepancies, our figures of coccidioidin positivity agree rather well with the known distribution of endemic areas of coccidioidomycosis.

Table I

Coccidioidin and Tuberculin Skin Tests

	Coccidioidin			Tuberculin (Purified protein derivative no. 2)			Tuberculin (Purified protein derivative no. 1)		
	Total cases	Positive	Per cent	Total cases	Positive	Per cent	Total cases	Positive	Per cent
San Joaquin Valley	156	98	62.8	27	22	81.4	20	3	15.0
San Francisco and Bay region	70	23	32.9	15	12	80.0	15	2	13.3
Arizona	250	70	28.0	38	29	76.3	30	4	13.3
Los Angeles and Southern California	615	99	16.1	100	80	80.0	134	20	14.9
New Mexico	60	7	11.6	12	10	83.3	9	1	11.1
Texas	14	5	35.7	1	1				
Total	1,165	302	25.9	193	154	79.7	208	30	14.4

Thirty-six patients upon whom skin tests had been performed were examined by autopsy. Twenty-five had reacted negatively to the coccidioidin skin test. Four of the negative cases showed no calcified lesions of the lungs. The lungs of 3 of the 25 had small calcified areas throughout the lung fields, suggestive of a healed miliary type of tuberculosis. The roentgenograms of the remainder of the negative reactors revealed calcified areas of different sizes in various parts of the lungs and in moderate numbers. In no instance was a single calcified lesion found. However, in most instances, one of the calcified areas was found to be larger than the remainder in the individual case. These areas of calcification varied from 4 to 20 in number and from 1 to 10 mm. in diameter. There was no uniformity of location except that the lesions were close to the walls of the bronchi or bronchioles and in peribronchial lymph nodes. In all probability most of these lesions were of tuberculous origin and were residual evidences of healed primary foci. All of the negative coccidioidin reactors had apical pleural scars except the 3 without post-mortem roentgenographic evidence of pulmonary calcification. These 3 cases constitute 12 per cent of the negative reactors. Of considerable interest is the fact that these three individuals were tuberculin negative.

The age limits of the negative coccidioidin reactors were from 38 to 82, with 19 of the 25 patients past 60 years.

Material from 7 of the negative reactors was injected into guinea-pigs. Material from 20 cases was cultured on two types of media. The animals and cultures were negative for *Mycobacterium tuberculosis*. This would indicate that calcified lesions in the higher age brackets are sterile regardless of their cause.

Spherules suggestive of *C. immitis* were found in the calcified lesions of one of the negative coccidioidin skin reactors. However, careful search of the histological material failed to reveal endosporulation essential to the differentiation of coccidioides from organisms of similar morphology known and unknown.

Two of the negative coccidioidin reactors were excluded from the negative series as both patients died of coccidioidal granuloma. One had typical Addison's disease with coccidioidal involvement of the adrenals. The other succumbed to a diffuse pulmonary involvement. These cases are examples of the well known fact that patients with progressive coccidioidal granuloma not infrequently react negatively to coccidioidin. Cultures and animal inoculations were positive in each case for *C. immitis*. The interesting feature of each case relevant to the subject of this paper was the finding of primary foci in the lungs and peribronchial lymph nodes of older duration than the advancing pulmonary lesions.

Case Histories of the Positive Coccidioidin Skin Reactors Seen at Autopsy

Case 1

A boy, age 15, from Bakersfield, California, entered the Santa Fe Hospital in December, 1939, complaining of chills, fever, loss of weight, and enlarging abdomen. These symptoms had been manifested periodically from August 1, 1939. He had a moderate anemia and a leukocytosis of 27,000 with 80 per cent polynuclear leukocytes. The coccidioidin skin test was markedly positive. At autopsy, a malignant hepatoma was found with metastases to the lungs. Three cm. below the right apex, close to the pleura, there was a small, fibrotic, encapsulated, calcified area of caseation 1.5 cm. in diameter. A smaller area of calcification was found in a peribronchial lymph node on the right side. Animal inoculations were not performed, but cultures were positive. Inoculations of the fungus culture into guinea-pigs failed to produce lesions. Sections of the lesions revealed a caseous area enveloped by a thick, fibrous-tissue capsule in which there was finely divided calcified material. Spherules and endospores were found. The lesions in the lymph node consisted almost entirely of fibrous tissue in which there was carbon pigment, calcium, and spherules of *C. immitis*.

Case 2

A white male, age 60, a section foreman, from Reedly, California, died on June 18, 1942, of arteriosclerotic and hypertensive heart disease. His coccidioidin skin test had been strongly positive on a previous hospital entry. Gross examination of the lungs revealed the presence of apical scars and many small areas of calcification in the tracheobronchial lymph nodes. In the upper anterior part of the left lower lobe there was an area of encapsulated caseation that had a diameter of 3 mm. The lesions were removed, sectioned, and injected into the testicles of guinea-pigs. One pig, injected with lesions from the lung, developed a testicular abscess which was negative for *C. immitis* and *Myco. tuberculosis*. Histological preparations of the pulmonary lesions revealed the presence of spherules but no endospores.

Case 3

A white male, age 65, a section foreman, from San Bernardino, California, died in the Santa Fe Hospital on January 29, 1941. Death was due to carcinoma of the prostate, osteosclerotic anemia, and terminal bronchopneumonia. Post-mortem roentgenograms of the lungs revealed areas of calcification in the peribronchial lymph nodes and multiple very small areas of calcification in both lower lobes. A larger area of encapsulated caseation with calcification was found in the upper anterior portion of the lower right lobe. Microscopical examination of the caseous area from the lung revealed the presence of many spores, shells of spores and a rare spherule filled with endospores. The lesion was typical of the healed lesion to be described. Areas of fibrosis were found in the peribronchial lymph nodes, but no *C. immitis* were demonstrated. Cultures from the primary lesion were negative. Animal inoculation was not made.

Case 4

A white male, age 60, a towerman, entered the hospital on January 22, 1942, and died on March 11, 1942. This patient was from Berkeley, California. Coccidioidin skin test was strongly positive. The cause of death was bronchopneumonia complicating carcinoma of the lung. Post-mortem roentgenograms of the lungs revealed fine areas of calcification and caseation, less than 1 cm. in diameter, in the lower right lobe, and one similar area of calcification in a peribronchial lymph

node on the right side. Representative areas were sectioned and ground up for cultures and animal inoculations. The sections showed areas of walled-off caseation in which there was little calcification. No spores of coccidioides were found and animal inoculations and cultures were negative for tubercle bacilli and *C. immitis*.

Case 5

A white American male, age 64, whose home was in Los Angeles, died in the Santa Fe Hospital of congestive heart failure resulting from hypertensive and arteriosclerotic heart disease. A coccidioides skin test was reported as weakly positive. The area of hyperemia was less than 1 cm. in diameter. A tuberculin skin test was negative. The autopsy revealed apical scars and pleural adhesions. Post-mortem roentgenograms of the lungs showed minute areas of calcification about 1 mm. in diameter in the lower part of the lower right lobe, a few similar areas in the upper part of the upper right lobe, and one such area in the left lower lobe towards the periphery. Two larger areas of calcification were found along one of the first branches of the right main bronchus. Two were confined to peribronchial lymph nodes. Microscopical examination of these areas revealed the presence of areas of caseation, silicotic nodules, and much fibrosis. Cultures and animal inoculations were negative for tubercle bacilli and *C. immitis*. The lesions were most likely of tuberculous origin.

Case 6

A white American male, age 63, a tailor, died on February 3, 1941, in the Santa Fe Hospital of complications resulting from carcinoma of the stomach. No skin tests had been performed. In the lower left lung, centrally located, there was a large area of encapsulated caseation. No apical scars or pleural fibrosis were noted. The area of caseation was surrounded by a thick, fibrous capsule in which there were a moderate number of round cells. Scattered throughout the caseous material at all levels there were large numbers of spores varying in size. In addition there were many large spores filled with endospores. Unfortunately, cultures and animal inoculations were not done. This is an example of a typical arrested pulmonary lesion of coccidioides. Twenty-five years ago this patient lived in Taft, California, for a period of several years. During the past few years he had returned to Taft for short visits. There was no history of upper respiratory infection or attacks of influenza in the years immediately preceding his death.

Case 7

The patient was a white male, age 60, whose home was in Gallup, New Mexico. Death was due to right cardiac failure, emphysema and chronic bronchitis. The lungs were placed in formalin before examination; therefore, no culture or animal inoculation was possible. Gross examination of the lungs revealed apical scars and thickening of the pleura. Post-mortem roentgenograms of the lungs showed three areas of calcification in the peribronchial lymph nodes on the left side, each about 1 cm. in diameter. In the base of the left lung there was a small area of calcification. These areas were excised and studied. In the section of the pulmonary lesion, which consisted of an oval area of caseation surrounded by a thick capsule, there were many oval bodies having central dense areas. No spherules were found in the lesions of the peribronchial lymph nodes.

Case 8

An American Negro, age 69, from Bakersfield, California, died in the Santa Fe Hospital of carcinoma of the stomach. The coccidioidin skin test was positive while the tuberculin skin test was negative. Five oval areas of calcification were found in the lower portion of the left lung and two similar areas in the upper right lobe. In a peribronchial lymph node on the left there was a rather large area of calcifica-

tion. Animal inoculations and cultures of the post-mortem material were negative. Sections of the calcified areas from the left lung contained an area of necrosis surrounded by a fibrous tissue capsule. A few spherules and a rare shrunken spore showing endosporulation were found.

Case 9

An American white male, age 65, a switchman, from Barstow, California, died in the Santa Fe Hospital of syphilis of the aorta and congestive heart failure. The coccidioidin skin test was positive. The tuberculin test was negative. Post-mortem roentgenograms revealed five areas of calcification in each lung. The largest had a diameter of 6 mm. Animal inoculations and cultures were negative for *C. immitis* and *Myco. tuberculosis*. Spherules, but no endospores, were found in one of the lesions of the lungs.

Case 10

A Negro male, age 54, a porter, whose home was in Los Angeles, died in the Santa Fe Hospital of congestive heart failure due to hypertensive heart disease. Coccidioidin skin test was positive. Post-mortem roentgenograms of the lungs showed one area of calcification at the base of the right lung, 0.5 cm. in diameter, and one at the base of the left lung, 0.3 cm. in diameter. A peribronchial lymph node in the left side contained a small area of calcification. Apical scars were not present. Spherules, but no endospores, were found in the pulmonary lesions. Animal inoculations and cultures were negative for *C. immitis* and *Myco. tuberculosis*.

Case 11

The patient was a white male, age 64, who lived in Bakersfield, California. Death was due to carcinoma of the tongue and terminal bronchopneumonia. Autopsy revealed obliteration of the pleural cavities by old fibrous adhesions. No apical scars were noted. Areas of calcification were found in the lower right lobe laterally and superiorly. Four peribronchial lymph nodes contained areas of calcification. Cultures and animal inoculation were negative for *Myco. tuberculosis* and *C. immitis*. No spores or endospores were found in the histological preparations.

Discussion

Dickson and others, on a number of occasions, have called attention to the similarity of coccidioidomycosis and tuberculosis. It is remarkable how this similarity prevails in the interpretation and reading of the skin tests and the appearances of the healed pulmonary lesions. As with the tuberculin test and tuberculosis, there is a rough quantitative relationship between the coccidioidin skin reaction and the activity or age of the primary coccidioides lesions. Moreover, a negative coccidioidin skin test may be obtained in the late stages of coccidioidal granuloma. Two such examples are given in Table IV; one patient dying of disseminated pulmonary coccidioidal granuloma and the other of Addison's disease due to coccidioides infection of the adrenals.

It is probable that persons with healed lesions of coccidioidomycosis may eventually lose their skin sensitivity to coccidioidin. One of the negative skin reactors had a calcified pulmonary lesion that contained spherules but no endospores.

There is no apparent cross-antigenic relationship of tuberculin and coccidioidin. This is apparent in Table II. Furthermore, a breakdown of 187 positive reactors to tuberculin (purified protein derivative, second strength) into geographic locations, as shown in Table I, shows no appreciable variation, whereas the positive reactors to coccidioidin are definitely localized to super-endemic areas.

The question of nonspecificity of coccidioidin was further studied by simultaneous skin tests of coccidioidin and antigens of other fungi. Antigens of Blastomyces, Aspergillus and Sporotrichum were so tested with completely negative results, as shown in Tables II and III. During the course of these investigations, a patient with blastomycosis of the scrotum was admitted to the service. He reacted negatively to coc-

TABLE II

Comparison of Coccidioidin and Tuberculin Skin Tests

Coccidioidin	Tuberculin	Total cases	Per cent
Positive	Positive	60	8.5
Negative	Positive	158	22.5
Positive	Negative	133	19.0
Negative	Negative	349	49.8
Total		700	

cidioidin in dilution of 1:1000, 1:100 and 1:10. Unfortunately, the patient left the hospital before we were able to have a blastomyces antigen prepared.

No clear clinical histories suggestive of coccidioidomycosis infections could be obtained from the positive reactors. This would suggest that subclinical infections are more common than the acute clinical phase of coccidioidomycosis. However, there are undoubtedly many unrecognized cases of coccidioidomycosis.

Calcified lesions of coccidioidomycosis were indistinguishable grossly from similar lesions of tuberculosis except that apical scars were not a part of the healed or arrested pulmonary phases of coccidioides. The primary disease resolves into one or more encapsulated fibrotic areas in various parts of the lung with associated single or multiple similar lesions in the peribronchial and tracheobronchial lymph nodes. These lesions in some cases were so small as not to be revealed on the roentgenograms taken during life. Furthermore, such lesions were easily missed unless roentgenograms were made of the lungs after removal from the thoracic cage. From examination of such films it becomes apparent that a single healed pulmonary lesion was a rarity. No doubt primary tuberculosis also resolves in multiple, rather than single, healed lesions of the parenchyma of the lungs.

The histological pattern was essentially that of tuberculosis except for the presence of the spherules. The centers were caseous and contained small calcified particles and faint outlines of necrotic lung tissue. Each lesion was surrounded by a dense, hyalinized, fibrous-tissue capsule, external to which there were collections of lymphocytes. In the older lesions the caseous material may be replaced by fibrous tissue. Located in the capsules and caseous material there were spherules of *C. immitis* and fewer large spherules containing endospores. Many of the organisms were shrunken, distorted, or ruptured. Varying amounts of calcification were noted in some of the spherules.

TABLE III

Comparison of Skin Tests with Coccidioidin and with Preparations of Other Pathogenic Fungi

Cases	Coccidioidin	Torula	Aspergillus
6	Positive	Negative	Negative
9	Negative	Negative	Negative

Cases	Coccidioidin	Blastomyces	Sporotrichum
8	Positive	Negative	Negative
18	Negative	Negative	Negative

Spherules were present in the healed lesions of 8 of the positive reactors. However, endosporulation was noted in only 5 of the 8 cases. We consider endosporulation essential in establishing the lesion as one of coccidioides. Inasmuch as budding was not demonstrated in any of these cases, we were inclined to accept the presence of spherules as presumptive evidence of healed coccidioidomycosis until the discovery by Emmons [9] of *Haplosporangium parvum*. However, 45.4 per cent of the 11 positive reactors seen at autopsy had proved healed lesions of coccidioidomycosis. Certainly this is a considerably higher figure than obtains in similar studies of the healed lesions of tuberculosis.

Considerable significance is attached to the fact that in none of the animals injected with material from the healed lesions was a lesion of coccidioides or tuberculosis demonstrated. Undoubtedly most of the healed lesions were of tuberculous origin. This would indicate that in healed tuberculosis, as well as healed coccidioidomycosis, the organisms are dead. Therefore, after a certain period it is unlikely that these lesions could be responsible for the disseminated and fatal forms of either disease. Moreover, the two fatal cases of coccidioidal granuloma (nos. 12 and 13, Table IV) were examples of slowly advancing, primary pulmonary forms with evidence of healing in the initial lesion.

TABLE IV

Positive Coccidioidin Skin Reactors: Cases Examined by Autopsy

Sex	Age	Skin reaction		Cause of death	Case no.	Guinea-pig inoculation	Cultures		Tissue findings	
		Coccidioidin	Tuberculin				*Coccidioides immitis*	Tubercle bacilli	Endospores	Spherules
M	15	Pos.	Neg.	Carcinoma, liver	1		Pos.	Neg.	Pos.	Pos.
M	60	Pos.		Heart disease	2	Neg.			Neg.	Pos.
M	65	Pos.		Carcinoma, prostate	3		Neg.	Neg.	Pos.	Pos.
M	60	Pos.		Carcinoma, lung	4	Neg.	Neg.	Neg.	Neg.	Neg.
M	64	Pos.	Neg.	Heart disease	5	Neg.	Neg.	Neg.	Neg.	Neg.
M	63	Pos.		Carcinoma, stomach	6				Pos.	Pos.
M	60	Pos.		Heart disease	7				Pos.	Pos.
M	69	Pos.	Neg.	Carcinoma, stomach	8	Neg.	Neg.	Neg.	Pos.	Pos.
M	65	Pos.	Neg.	Heart disease; syphilis	9	Neg.	Neg.	Neg.	Neg.	Pos.
M	54	Pos.	Neg.	Heart disease	10	Neg.	Neg.	Neg.	Neg.	Pos.
M	64	Pos.	Neg.	Carcinoma, tongue and prostate	11	Neg.	Neg.	Neg.	Neg.	Neg.
M	45	Neg.	Neg.	Coccidioidal granuloma, pulmonary	12	Pos.	Pos.	Neg.	Pos.	Pos.
M	60	Neg.	Neg.	Addison's disease, due to coccidioidal granuloma of adrenals	13	Pos.	Pos.	Neg.	Pos.	Pos.

Blank spaces = tests not performed.

Conclusions

1. The incidence of positive coccidioidin skin tests in the Santa Fe Coast Lines Hospital is 25.9 per cent.

2. No cross-antigenic relationship was demonstrated between tuberculin and coccidioidin.

3. Skin antigens of other fungi prepared in a manner similar to that for coccidioidin produced no skin reactions in patients reacting positively to coccidioidin.

4. A case of cutaneous blastomycosis reacted negatively to the coccidioidin in several dilutions.

5. As to its clinical significance, the coccidioidin skin test is to be evaluated in the same manner as the tuberculin skin test.

6. Autopsies revealed healed calcified lesions in the lungs of all positive coccidioidin skin reactors. Grossly, the healed primary pulmonary lesions of coccidioidomycosis are indistinguishable from similar lesions of tuberculosis.

7. Spherules and endospores of *C. immitis* were found in 45.4 per cent of the positive reactors. Spherules, but no endospores, were found in three additional cases of the positive coccidioidin group.

We are greatly indebted to the internes of the Santa Fe Hospital, and particularly to Dr. Otto Lange, for their help in performing the skin tests. We are extremely appreciative of the aid extended by Dr. Charles E. Smith of Stanford University School of Medicine.

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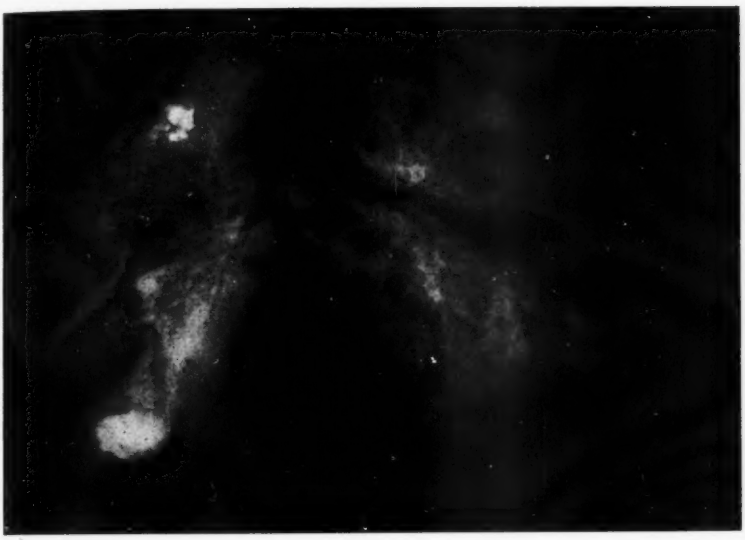
DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Case 1. Post-mortem roentgenogram of lungs. Area of calcification in upper right lobe, healed coccidioidomycosis. Smaller areas of calcification in right peribronchial lymph nodes. Other shadows are due to metastatic malignant hepatoma.

FIG. 2. Case 1. Lesion found in right upper lobe. × 20.

FIG. 3. Case 1. Peribronchial lymph node with spherules present in the center of an area of fibrosis. × 100.



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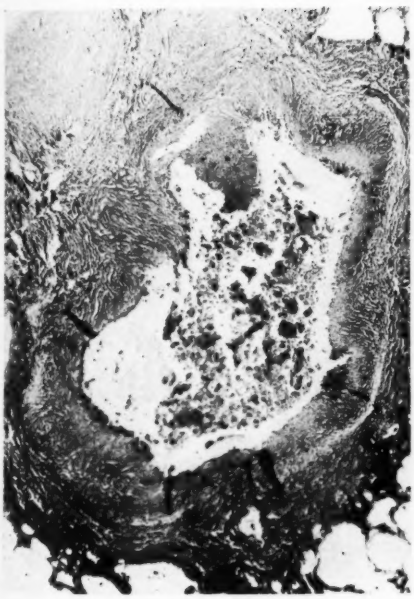

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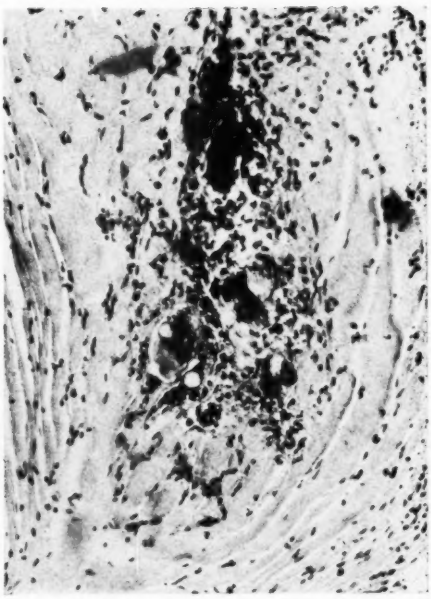


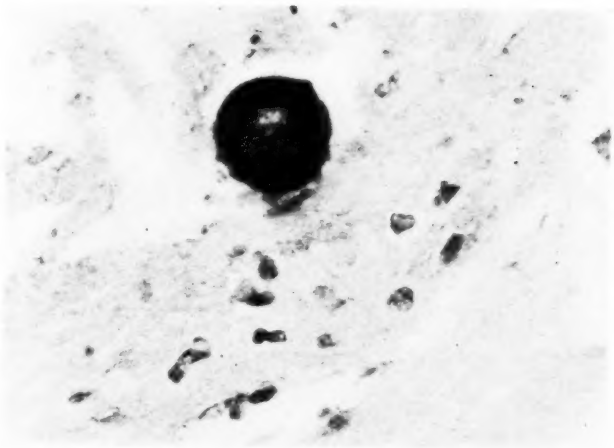
3

Butt and Hoffman Pulmonary Coccidioidomycosis

PLATE 83

FIG. 4. Case 1. Shrunken, partly calcified spherule with endosporulation. × 1200.

FIG. 5. Case 1. Spherules of *Coccidioides immitis* in a peribronchial lymph node. × 1200.



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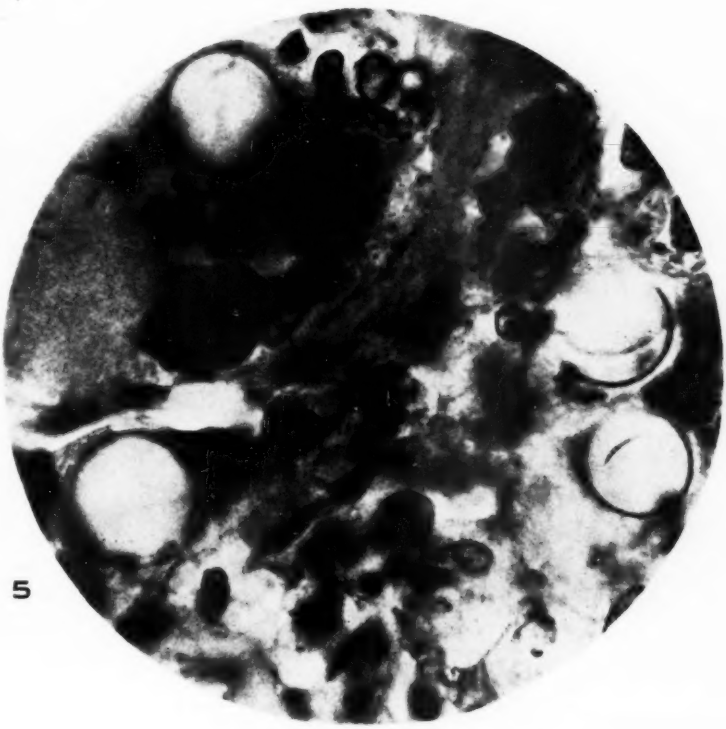

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Butt and Hoffman Pulmonary Coccidioidomycosis

PLATE 84

FIG. 6. Case 3. Area of calcification in hilar region of right lung. Smaller areas of calcification in lower left lobe and peribronchial lymph nodes.

FIG. 7. Case 3. Lesion in hilar region of right lung. × 100.

FIG. 8. Case 3. Lesion of right lung showing spherules and spherules with endospores. × 450.

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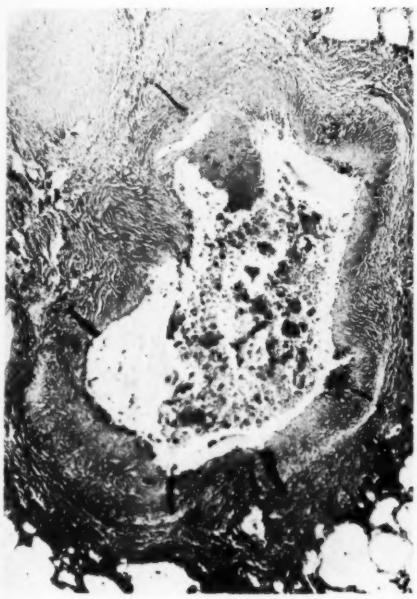

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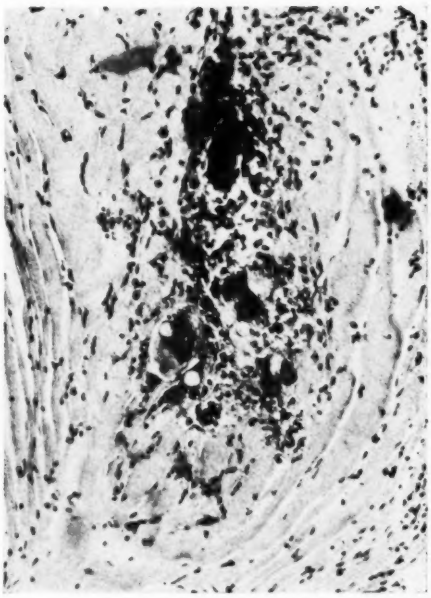

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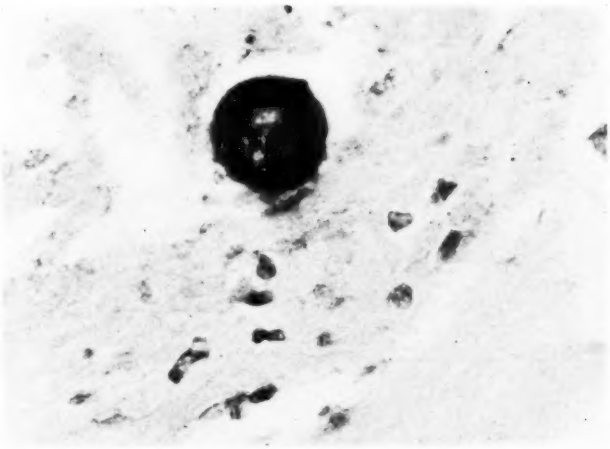
Butt and Hoffman Pulmonary Coccidioidomycosis

PLATE 83

FIG. 4. Case 1. Shrunken, partly calcified spherule with endosporulation. × 1200.

FIG. 5. Case 1. Spherules of *Coccidioides immitis* in a peribronchial lymph node. × 1200.



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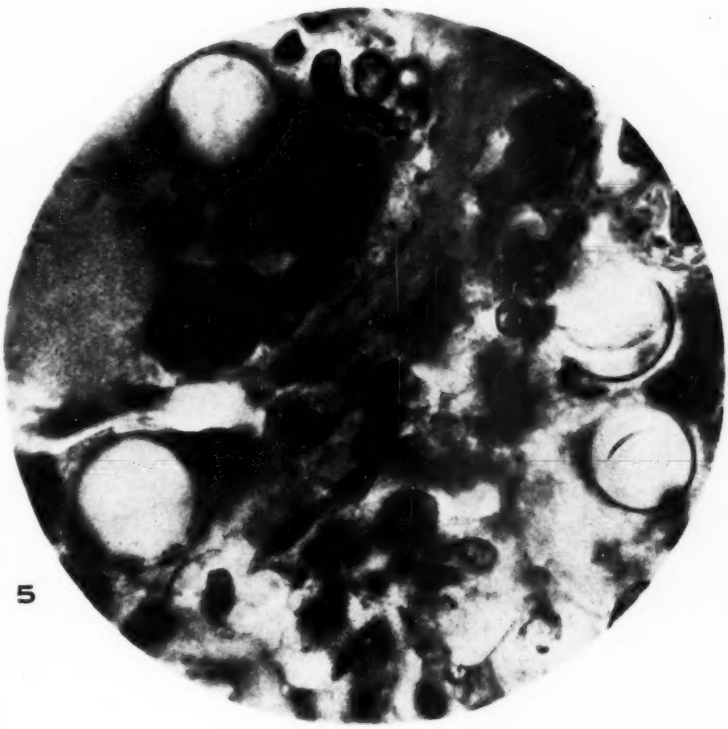

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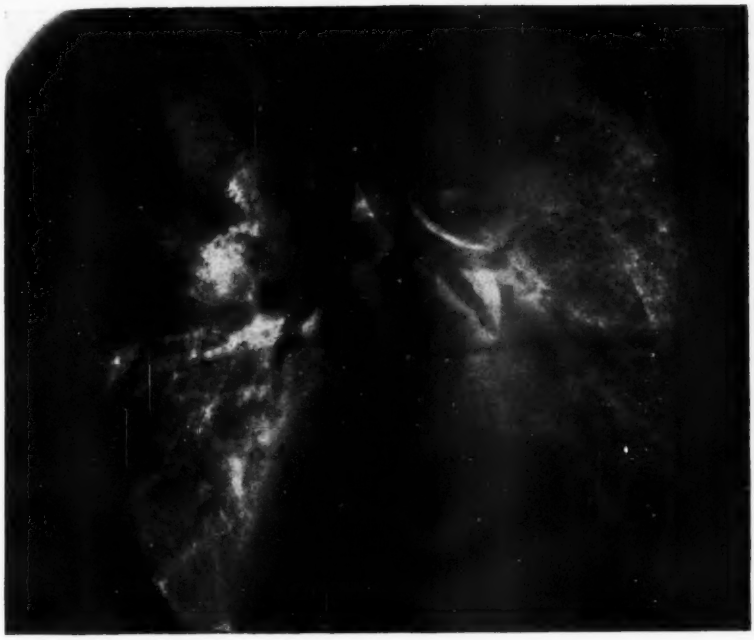
Butt and Hoffman Pulmonary Coccidioidomycosis

PLATE 84

FIG. 6. Case 3. Area of calcification in hilar region of right lung. Smaller areas of calcification in lower left lobe and peribronchial lymph nodes.

FIG. 7. Case 3. Lesion in hilar region of right lung. × 100.

FIG. 8. Case 3. Lesion of right lung showing spherules and spherules with endospores. × 450.



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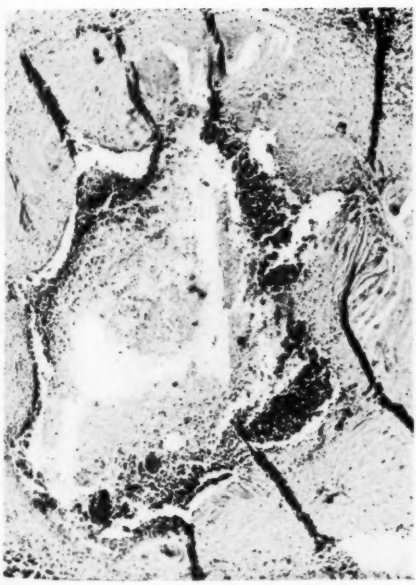

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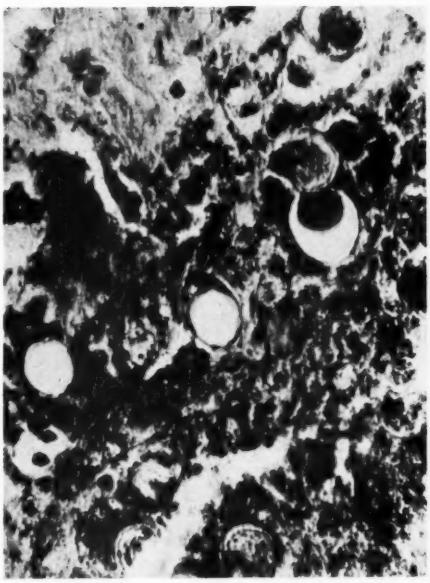

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Butt and Hoffman

Pulmonary Coccidioidomycosis

PLATE 85

FIG. 9. Case 3. Peribronchial lymph node showing fibrotic oval mass containing shrunken pink-staining spores and carbon pigment. × 22.

FIG. 10. Case 6. Deformed spherule of *Coccidioides immitis* with endospores. × 1080.


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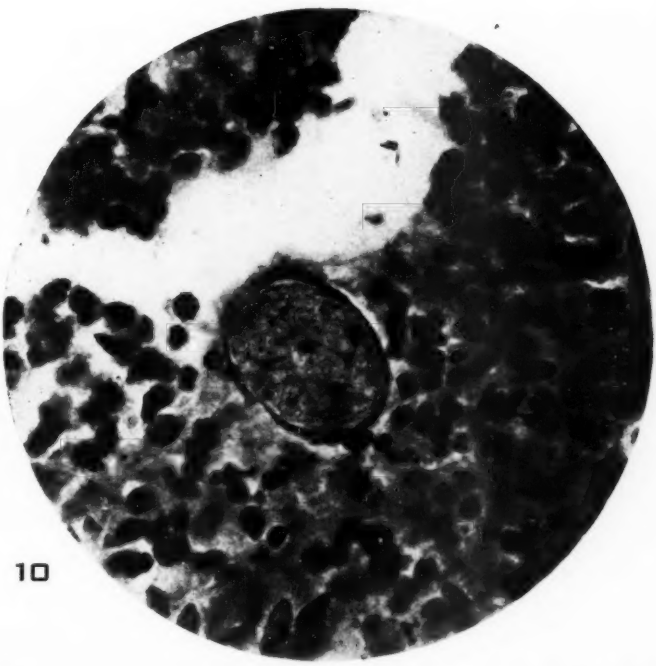
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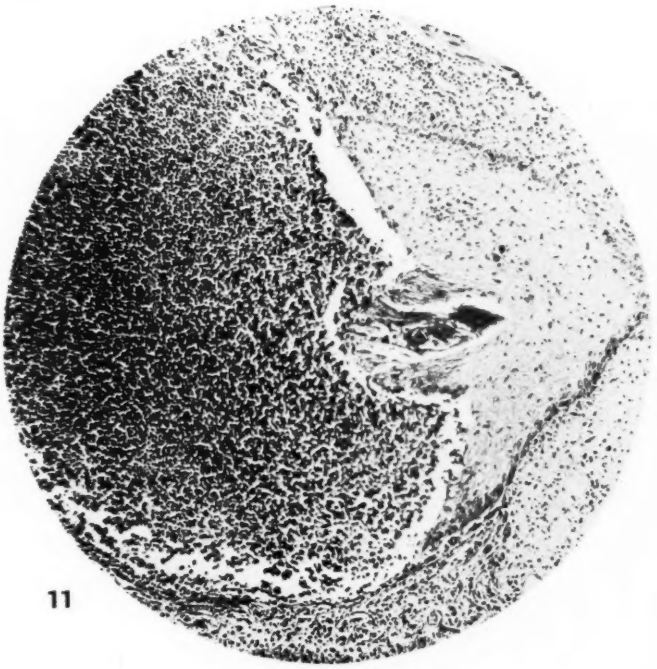
PLATE 86

FIG. 11. Cutaneous blastomycosis. Coccidioidin skin test was negative. × 90.

FIG. 12. Cutaneous blastomycosis showing budding organisms. × 1080.



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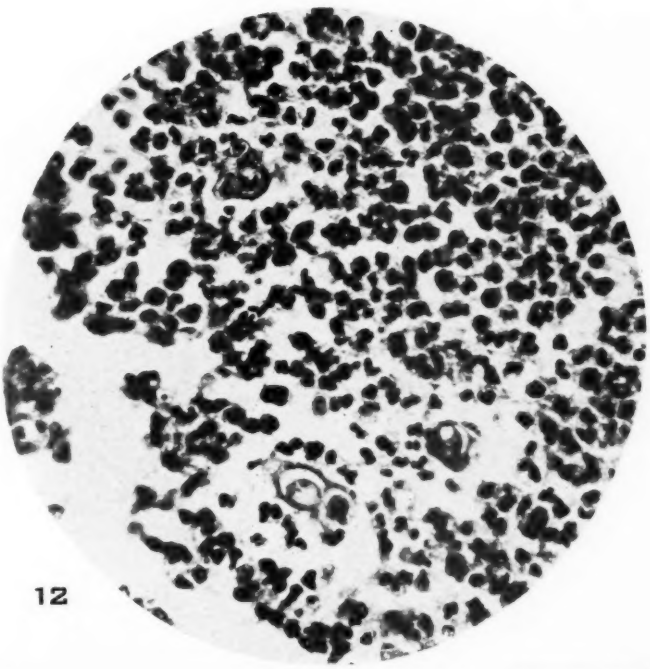

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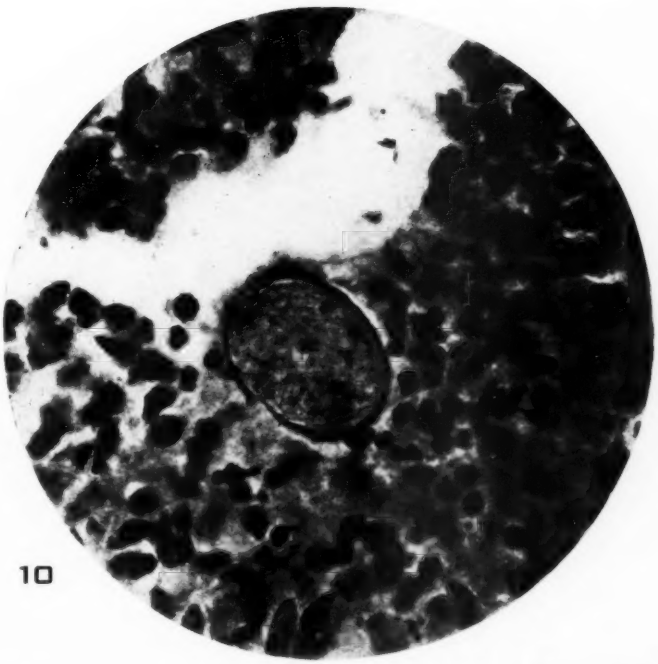
Butt and Hoffman Pulmonary Coccidioidomycosis

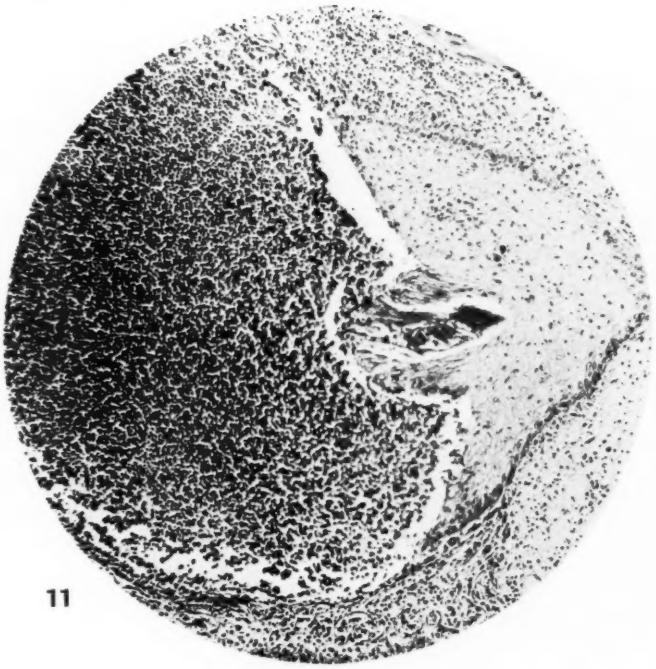


Butt and Hoffman Pulmonary Coccidioidomycosis

PLATE 86

FIG. 11. Cutaneous blastomycosis. Coccidioidin skin test was negative. × 90.

FIG. 12. Cutaneous blastomycosis showing budding organisms. × 1080.



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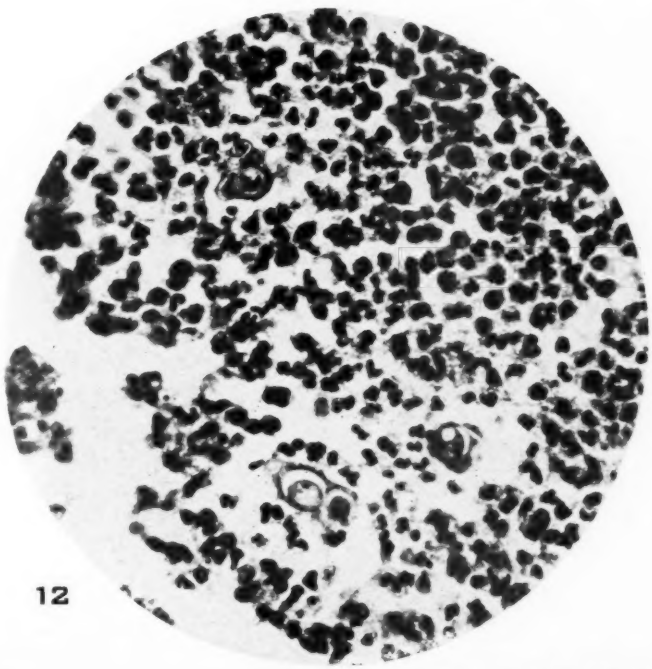

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Butt and Hoffman Pulmonary Coccidioidomycosis

SUBACUTE BACTERIAL (STREPTOCOCCUS VIRIDANS) PULMONARY ENDARTERITIS *

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Clinical cases in which *Streptococcus viridans* has produced vegetations on the intima of the pulmonary artery are infrequent. Such lesions have been encountered under various conditions.

Vegetations in subacute bacterial endocarditis characteristically extend from their original location on the valves to the surrounding mural endocardium. Similarly, this tendency results in spread from the pulmonary semilunar valves for some distance up the pulmonary artery. One may assume that this mechanism prevails in the cases presenting a vegetative lesion involving, in continuity, both the semilunar valves and the stem of the pulmonary artery. These cases occur comparatively frequently. They usually show vegetations also on the valves of the left side of the heart. Old rheumatic lesions are commonly present upon which the bacterial process is superimposed.

Isolated vegetations situated in the pulmonary artery above the valves are associated with pathological lesions of a varied nature. These lesions are thought to account for the unusual localization of the vegetations, and of them congenital defects in the heart and great vessels are the most important. Patent ductus arteriosus is the most common deformity in this group. Abbott [1] found that 21 of 92 patients with patent ductus arteriosus died with subacute bacterial endarteritis superimposed on the defect. The vegetations developed in the pulmonary artery adjacent to the ductus where the arterial wall is subjected to the constant impact of the blood which is forced from the aorta into the pulmonary artery. Other congenital lesions are occasionally associated with *Str. viridans* infection in the pulmonary artery. This is illustrated by a case with congenital interventricular septal defect, persistent conus arteriosus, and bicuspid pulmonary valve reported by Posey.[2]

A variety of pathological processes other than congenital deformities may also predispose to the nidation of *Str. viridans* in the pulmonary artery. These, however, are very infrequent. An example is the case reported by Marchal, Porge, and Ortholan [3] of an aortic aneurysm impinging on the pulmonary artery.

Mehlin's [4] first case of *Str. viridans* infection, in a man, 25 years old, presented a vegetation which was confined to the stem of the pul-

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monary artery. The wall of the vessel was otherwise normal. The pulmonary semilunar valves, however, were thickened and rigid, confirming the clinical diagnosis of pulmonary insufficiency which was made several years before death. The myocardium also contained numerous small scars. The lesions of the pulmonary cusps and the myocardium were apparently the result of rheumatic disease. Though the wall of the pulmonary artery was thought to be normal, except for the vegetation, one will recall the changes occurring with rheumatic fever in the pulmonary artery as described by Kugel and Epstein.[5] It may be suspected, therefore, that previous involvement, though unnoticed, was responsible for the localization of the vegetation in the pulmonary artery in Mehlin's first case.

The literature contains several reports of cases of isolated vegetations in the pulmonary artery that are impossible to evaluate since no bacteriological studies were made. Such instances are Mehlin's third case, the second case in Reiche's [6] series (1892), and Oberndorfer's [7] two cases (1918). The cases of Reiche and Oberndorfer were reviewed by Mehlin.[4] An analysis of the available data, however, makes it appear improbable that they were caused by *Str. viridans*.

In reviewing the literature, no instance of isolated vegetations in the pulmonary artery above the semilunar valves caused by *Str. viridans* has come to my attention in which both predisposing changes in the wall of the pulmonary artery and pathological changes in the heart and aorta were absent. However, this situation prevailed in the following case.

Report of Case

W. P., Unit no. 42433. A white girl, 4½ years old, was admitted on November 20, 1943, with the history of a prolonged fever.

Past History. The child had had occasional colds. She had been immunized against diphtheria.

Family History. The information obtained concerning the family history was noncontributory.

Present Illness. The child had been sick with fever and anorexia for about 7 weeks. Two weeks after the onset, the patient's left knee became tender but was not swollen. The tenderness persisted for 10 days, then the large joints of the right upper extremity became similarly involved. The patient had severe sweats and frequent epistaxis during the 2 weeks prior to admission. Antimalarial treatment and sulfa drugs were without benefit.

Physical Findings. The patient was acutely ill and poorly nourished. The skin and mucous membranes were pale. The rectal temperature was 104.4° F. The pulse was rapid and bounding. A cough was present and coarse râles were heard throughout the lungs. The apex beat of the heart was immediately lateral to the midclavicular line. A systolic murmur was heard in the pulmonic area and the second pulmonic sound was thought to be accentuated. The spleen was enlarged. The right wrist, elbow, and shoulder were tender but not swollen.

Laboratory Findings. The red blood cell count was 1.59 million per cmm. and the hemoglobin was 4½ gm. per cent. The white blood cells numbered 11,750 per

cmm., with the following differential count: juveniles, 2 per cent; stabs, 6 per cent; segmented polymorphonuclear leukocytes, 77 per cent; lymphocytes, 14 per cent; monocytes, 1 per cent. Urinalysis revealed 1 plus albumin; red blood cells and casts were not found. Several large areas of consolidation in the periphery of the lower portion of the right lung were demonstrated by a roentgenogram. Blood culture yielded *Str. viridans*.

Clinical Course. The patient grew progressively worse during the 7 days of hospitalization. Her cough increased in severity. The respirations were labored and their rate increased to 50 per minute. Her pulse rate was 160 per minute. The temperature was intermittent in type, ranging between 98° and 105° F. There was some bleeding from the rectum. Slight edema of the face and ankles was present on the day of death.

Autopsy Findings

The autopsy was performed 6 hours after death. The following significant pathological changes were present:

The *heart* presented small areas of fibrin on the epicardium. The pericardial sac contained 50 cc. of clear fluid. All of the chambers of the heart were dilated, those on the right more than those on the left side. All valves were delicate and translucent. The mural endocardium glistened. The chordae tendineae were grossly normal. The aorta appeared intact. Histological sections from the heart failed to reveal any significant pathological changes.

The *pulmonary artery* showed one large and several small vegetations (Fig. 1). The large vegetation arose with a broad base from the right side of the pulmonary artery at the level of its division into the right and left main branches. It measured 2 cm. in its widest diameter and extended for 1 cm. into the right main branch. An ulceration of the vessel wall could be seen only after removal of the firmly attached vegetation. The arterial wall was thickened at the site of the lesion. *Str. viridans* was cultured from this vegetation. Several vegetations measuring 2 to 3 mm. were found on the left side of the pulmonary artery. The most proximal of these was situated 2 cm. above the semilunar valves, while the most distal one was found in the left main branch 2.5 cm. above the point of division of the pulmonary artery. The lesions were separated from each other by normal appearing intima and vessel wall. The circumference of the pulmonary artery, determined 2 cm. above the semilunar valves, exceeded the circumference of the aorta at a corresponding level by 2 mm., which is a normal finding. No congenital malformation was found.

Sections were taken through both the large and the small vegetations. Since the latter lesions represented a less advanced stage of involvement, their histological appearance is described first. They consisted almost entirely of gram-positive cocci, often distinctly arranged in chains. Weigert's stain revealed a destruction of the elastic lamellae

at the base of these vegetations (Fig. 4). This change was most severe near the bacteria, concentrically decreasing in severity toward the periphery. Faintly staining remnants of elastic tissue were present within the vegetation. The destruction of the inner layers of the arterial wall was accompanied by a considerable inflammatory reaction (Fig. 2). The inflammatory cells extended into the adventitia. Polymorphonuclear leukocytes were more numerous, though not abundant, in the vicinity of the vegetation while in the more distal areas lymphocytes and macrophages predominated. Numerous fibroblasts and capillaries were present at the base of the vegetation. The vasa vasorum did not reveal any significant changes.

Microscopical sections through the large vegetation showed extensive destruction of the underlying arterial wall as illustrated by the elastic tissue stain. The infiltration with inflammatory cells was similar in location to, but more extensive than, that encountered in the small vegetation. The fibroblastic proliferation was conspicuous at the base of the vegetation, where occasional giant cells also were found (Fig. 3). An attempt at organization of the vegetation was noted. Bacteria were few in number and they were confined to the area of the vegetation near the lumen. In addition to the diffuse inflammatory infiltration, focal accumulations of inflammatory cells were seen in the deeper layers of the wall.

The *right lung* showed numerous large, dark red, raised, firm, wedge-shaped areas in the periphery of the lower and middle lobes. Central softening of these lesions with the formation of a small amount of purulent material was noticed in two instances. Thrombi or emboli were present in the blood vessels near the apices of some of the infarcts. A patchy consolidation of the remainder of the right lower lobe was found. Numerous bronchioles contained purulent exudate. Microscopical sections from the right lung revealed areas where all the alveoli were filled with red blood cells. Necrosis of infarcts was observed but the areas of necrosis were largely walled off by great numbers of fibroblasts growing out with abundant capillaries. The paucity of leukocytes in the necrotic focus as well as in the surrounding tissues was conspicuous. The *left lung* failed to reveal any infarcts. There were patchy areas of consolidation in the dependent portions and a purulent exudate was seen in numerous bronchioles. Groups of alveoli and bronchioles filled with inflammatory exudate were found in sections from both lungs.

The *spleen* weighed 218 gm. It was moderately soft and the follicles were swollen. Microscopically, the splenic pulp was hyperplastic. The *liver* was enlarged, grayish, and friable. The microscopical sections

revealed small fat globules in the liver cells and an infiltration of lymphocytes about the portal spaces. The *kidneys* were pale and flabby. Histologically, there was some cloudy swelling of the epithelial cells in the tubules. The glomeruli were intact.

Pathological-Anatomical Diagnoses. Subacute bacterial (*Str. viridans*) pulmonary endarteritis; serofibrinous pericarditis; multiple septic infarcts in right lung; moderate hyperplasia of the spleen; parenchymatous degeneration of the liver and kidneys; bronchopneumonia, bilateral.

Discussion

It may be thought that, upon closing, the ductus arteriosus had left a defect in the arterial wall on which the vegetation became superimposed. This supposition was untenable, however, since the large vegetation arose from the right side of the stem of the pulmonary artery and from the adjacent part of the right main branch. The ductus arteriosus links either the left circumference of the stem of the pulmonary artery or its left main branch with the aorta.

Allen [8] analyzed the nature of the vegetations in bacterial endocarditis with the aid of differential connective tissue stains. He observed that the bulk of the vegetations, protruding into the lumen, was formed by necrotic tissue derived from the involved valve. Thrombotic material contributed to the formation of the vegetation to a much smaller extent, according to Allen. The histological structure of the vegetations in the present case confirms Allen's observations, which seem to apply to mural vegetations as well as to those on valves. Elastic tissue stains from a small vegetation revealed that a large part of the projecting mass was composed of tissue derived from the arterial wall, as demonstrated by the faintly staining remnants of elastic tissue in the amorphous material (Fig. 4). It is suggested that marked localized swelling of the part of the wall exposed to the bacterial action causes it to bulge into the lumen. After this tissue has become necrotic and amorphous, it is difficult to differentiate it from a thrombus. The fibroblastic proliferation which extends from the base into the necrotic zone also contributes to the growth of the vegetation.

Since a wide area of normal vessel wall separated the vegetations, each may be considered to have developed independently. The small vegetations presented the appearance of early lesions. The destruction of the vessel wall proceeded concentrically from the intima outward. The maximal involvement was found in the most superficial portion of the intima. No changes were observed in the vasa vasorum. Therefore, it appears that the vegetations had their origin in the vascular endothelium.

These observations on the development of the vegetations are supported by the recent experimental studies conducted by Mac Neal and his associates.[9-11] In the early stages of *Str. viridans* infection of the rabbit, they found a widespread involvement of the endothelial cells. The infection tended to progress on the auricular surfaces of the mitral and tricuspid valves and on the ventricular surface of the aortic cusps. Mac Neal and associates stress that the diffuse invasion of the endothelial cells by the streptococci was by no means limited to the heart but could be found in the endothelial cells lining vascular channels elsewhere. If these changes also occur in man, an initial, potential lesion may be assumed to occur frequently in the pulmonary artery during the earliest stages of *Str. viridans* infection. If so, the initial lesions must usually heal with complete restitution.

Mechanical factors like the impact of blood, friction of approximating endothelial surfaces, contact with a larger amount of bacteria-laden blood, and pre-existing pathological changes are all known to be factors in the establishment of the vegetations in subacute bacterial endocarditis. There is also a great preponderance of left-sided cardiac involvement in this disease as illustrated by Libman's [12] finding of only one case with a lesion on the right side of the heart in a series of more than 100 cases of subacute endocarditis. On the other hand, the lesions caused by the more aggressive organisms in acute endocarditis occur on the right side of the heart more commonly. Libman found involvement of the right side of the heart in 26.8 per cent of his cases of acute endocarditis. These bacteria localize more often in hearts which have apparently not been involved previously, and they show some tendency to establish lesions in locations where the conditions of increased mechanical stress do not exist.[13]

In the present case, the lesions developed in the lesser circulation, involved an apparently healthy vessel wall, and occurred in an atypical location where undue mechanical stress could not be postulated. The *Str. viridans* localized, therefore, in a manner resembling that of the aggressive organisms encountered in acute endocarditis. Perhaps this *Str. viridans* possessed unusual virulence. This assumption also seems supported by the fairly rapid course of the disease which led to death 8 weeks after the first symptoms were noticed.

Str. viridans rarely produces an acute endocarditis (Held and Goldbloom [14]). Although one may be tempted to place the present case in this category, there are both clinical and pathological differences to be noticed between my case and those designated as acute endocarditis. This is illustrated by a comparative study of acute and subacute *Str. viridans*

endocarditis conducted by Held and Goldbloom.[14] They found the acute cases to be characterized clinically by a readily demonstrable acute infection which preceded the endocarditis. There was also a lack of anemia and a paucity of embolic phenomena. Pathologically, the lesions on the valves were predominantly ulcerative. In the present case, there was no history of preceding acute infection. The portal of entry of the infection was obscure, as it commonly is in subacute bacterial endocarditis. The anemia was profound. Due to the localization of the large vegetation in the beginning of the right main branch of the pulmonary artery, emboli could reach only the right lung and the latter was studded with infarcts. The slower progression of lesions due to *Str. viridans* was manifested by the marked fibroblastic reaction surrounding the infarcts and by the paucity of leukocytes. The arterial lesion was characterized by the formation of vegetations. Ulceration was apparent only after the vegetation had been removed. The marked proliferation of fibroblasts and the presence of occasional giant cells at the base of the vegetation also conformed to the type of lesion encountered in subacute bacterial endocarditis. The septic type of temperature in this case occurred at a time when pulmonary infarcts were present. It is not unusual to observe fever of this type in advanced cases of subacute bacterial endocarditis which are complicated by emboli.

Summary

A case of *Streptococcus viridans* endarteritis of the stem of the pulmonary artery is reported. There were no changes found in the heart and great vessels to account for the atypical localization of the vegetations.

The vegetations in the pulmonary artery consisted, to a large extent, of necrotic tissue arising from the vessel wall. This is in accordance with the observation that the vegetations in endocarditis are derived largely from tissue of the valves. Observations in this case suggest the inception of the vegetative lesions from the endothelium.

The atypical localization of the vegetations resembles that of the lesions encountered with the more aggressive organisms of acute endocarditis. This suggests an unusual virulence of the *Str. viridans* in this case. However, the appearance of the lesion and other important clinical and pathological findings are those usually found with subacute bacterial endocarditis.

The rarity of atypical pathological processes of the kind reported here emphasizes the importance of mechanical stress and of predisposing lesions for the localization of *Str. viridans*.

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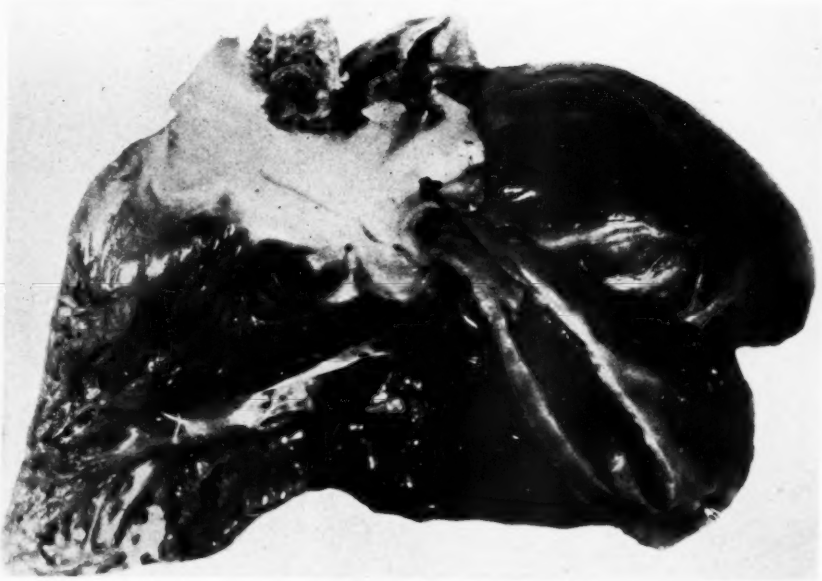
DESCRIPTION OF PLATES

Plate 87

Fig. 1. A large vegetation is shown to arise on the right side of the pulmonary artery at the level of its division and extends a short distance into the right main branch. Several small vegetations are present on the left side of the pulmonary artery and in its left main branch as indicated by the arrow.

Fig. 2. An inflammatory reaction is present at the base of a small vegetation. It is most intense adjacent to the bacterial mass and decreases toward the periphery. Hematoxylin and eosin stain. × 100.

1



2

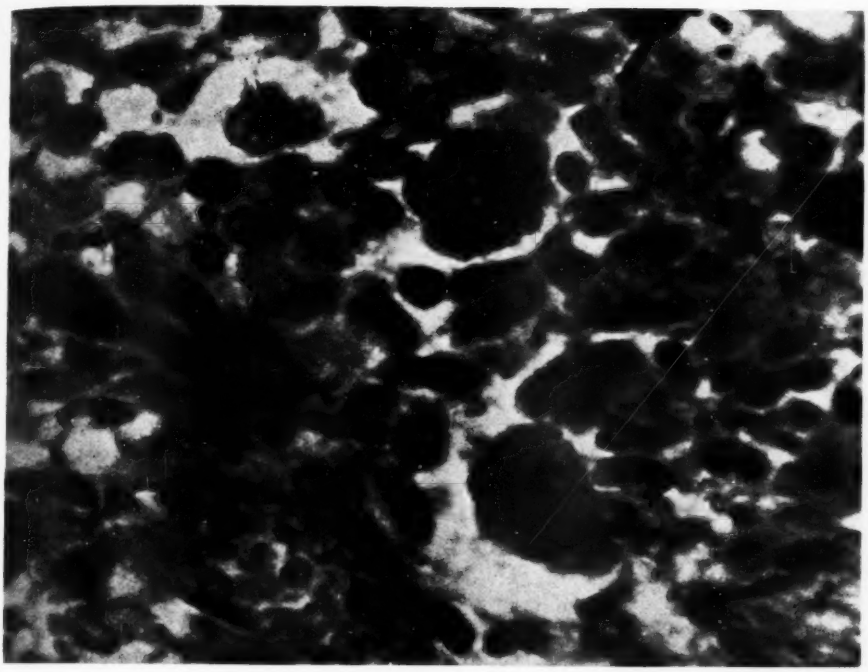
Rhoden Subacute Pulmonary Endarteritis

PLATE 88

FIG. 3. Giant cells are present among the fibroblasts at the base of the large vegetation, a finding often encountered with the lesions of subacute bacterial endocarditis. Hematoxylin and eosin stain. × 450.

FIG. 4. The destruction of the elastic fibers at the base of a small vegetation is shown. Faintly staining remnants of elastic tissue within the vegetation suggest that the latter consists largely of tissue derived from the wall of the vessel. Weigert's elastic tissue stain. × 100.

3



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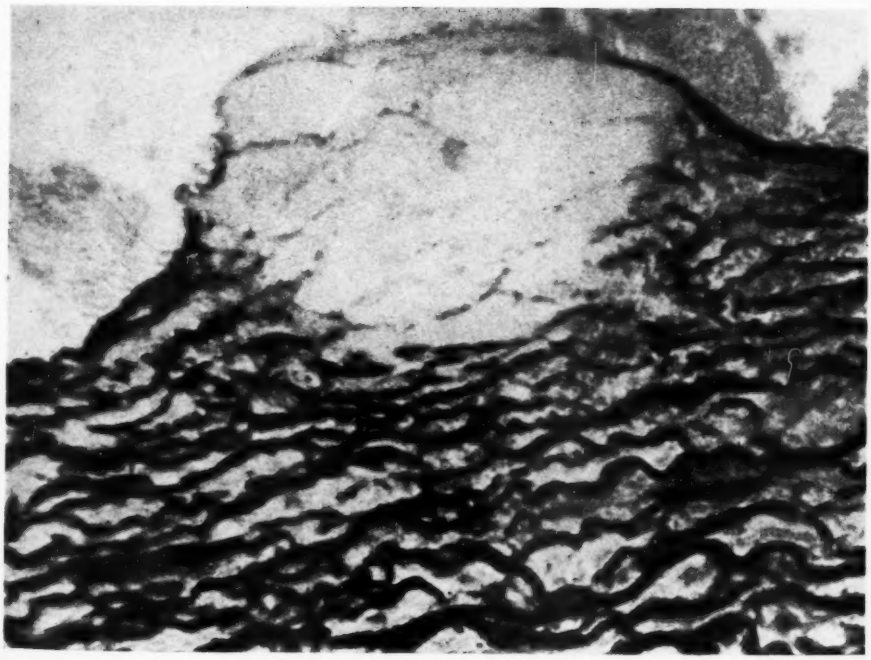

Rhoden Subacute Pulmonary Endarteritis

ACUTE DIFFUSE DEMYELINATING ENCEPHALOPATHY

Report of Two Cases *

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Considerable interest in diffuse demyelinating disease of the brain has arisen in recent years, not only in relation to its own etiology but also because of possible light which its study may cast upon its more chronic companion disease, multiple sclerosis, and to facilitate its clinical diagnosis. Following the original descriptions of the disease under a variety of clinical syndromes (encephalitis periaxialis diffusa, aplasia axialis extracorticalis congenita, symmetrical cerebral central lobar sclerosis, encephalopathia scleroticans, etc.[1]), new cases were reported in these sundry small categories, with many fine shades of distinction often becoming necessary in this system of classification.

An excellent descriptive study was contributed in 1924 by Collier and Greenfield.[2] Next came the statement in 1928 by Globus and Strauss [3] that at least a large group of these diseases could be considered as purely degenerative and therefore should be segregated under the heading of progressive degenerative subcortical encephalopathy. Their criterion for purely degenerative cases was absence of any cellular inflammatory reaction other than mild perivascular infiltration and gitter cell formation incidental to the clearing away of débris from dying cells. When Gasul [4] surveyed the literature in 1930 he found 72 cases (the number has risen rapidly since then). He concluded from that study that not all cases were purely degenerative, and that the etiology probably involved multiple factors.

From the clinical standpoint, meanwhile, Bouman [5] concluded, from a complete study of all available cases in the literature up to 1934, that there was no single group of cases with sufficient similarities among themselves and differences from other cases to be segregated as a distinct subdivision of diffuse sclerosis.

Finally, Ferraro [1] presented a rather exhaustive historical, clinical, pathological, and experimental study to show that all primary (and even secondary) demyelinating diseases of the central nervous system were essentially the same. He included multiple sclerosis, encephalitides following acute exanthemata, human and experimental deficiency conditions, and diseases following injection of such experimental toxins as potassium cyanide, tetanus toxin, and normal brain emulsion. As the situation stands today, most writers seem willing to accept the unifica-

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tion of at least the acute diffuse demyelinating diseases, if not of all demyelinating diseases. Papers are still being presented under one or another of the old syndromic titles, however.

Basis for Classification

The need for unification, at least for diffuse cerebral sclerosis, becomes apparent if one considers that:

1. "Specific" symptoms and neurologic signs may be produced by any of the diseases and depend only upon the rapidity, completeness, and anatomic location of the pathologic process. As indicated by Ferraro,[1] this point is fairly well accepted.

2. No constant difference in the above factors, and their resulting symptoms, can be found in the familial cases, in cases of any particular age group, or in any other subdivision of diffuse sclerosis. Disseminated sclerosis, of course, occurs in a decidedly older age group and with a more extensive and notoriously patchy distribution throughout the nervous system.

3. The pathologic findings are the same in all demyelinating diseases, as shown by Ferraro,[1] and the amount of terminal gliosis versus cyst formation and liquefaction, of axon cylinder preservation versus complete destruction, etc., depends upon the rapidity and severity of the process in the individual case rather than upon membership in one or another clinical syndrome.

4. Acute forms of cerebral multiple sclerosis are clinically and pathologically often indistinguishable from encephalitis periaxialis diffusa, as pointed out by Ferraro,[1] Meyer and Pilkington,[6] Reeves and Anderson,[7] and others.

5. *"Acute diffuse* sclerosis of *childhood"* may occur at *any age*,[1, 8, 9] may have *patches* anywhere in the central nervous system and involve gray matter as well as white,[1, 7] and may last as long as *15* years.[10] Thus it is difficult to delimit sharply so-called Schilder's disease from multiple sclerosis, when clinical as well as pathologic differences may disappear.

Etiology

It is significant that, as already implied, the demyelinating diseases with known etiology may be indistinguishable pathologically from those comprising the problem groups. Thus Ferraro [1] includes the demyelination of pernicious anemia, of bacterial toxins, of inorganic poisons, and of avitaminosis, in his system of classification, side by side with the syndromes of unknown etiology. Therefore it would seem unduly speculative for one to say that encephalitis periaxialis diffusa is inflammatory, or is degenerative, or even that there is but a single etiologic

factor. Indeed, it is more likely, by inference from the above facts, that the demyelinating diseases have a variety of causes and are thrown together because of a similarity in pathologic changes. When the various etiologic factors become known we shall have a satisfactory basis for classification; such a basis is of value in regard to prevention, treatment, and prognosis, all of which are of ultimately greater importance than fine distinctions in diagnosis.

A perusal of possible etiologic factors in individual cases of diffuse sclerosis does not clarify the situation. The experimental agents already mentioned (potassium cyanide, tetanus toxin, brain emulsion) have nothing in common with each other nor with canine distemper, human post-exanthematous encephalitis, vitamin deficiencies, nor with others listed below. Mackay [10] reported two cases following bacillary dysentery and pneumonia; Jervis and Kindwall [9] reported a case in an adult following severe chronic ergotamine poisoning. Winkelman and Moore [8] listed birth trauma, avitaminosis, acute infections, tuberculosis, adrenal atrophy, and carbon monoxide among causes reported or suggested in the literature. Several authors report a mild upper respiratory infection preceding the acute onset of symptoms, as in case 2 presented here. Diphtheria toxoid, administered to this patient, was followed by a convulsion within 2 hours, and it is difficult to conceive of its being the cause. In case 1 of this paper there was possibly an ascending infection, with chronic organizing ependymitis and fibrosis of the choroid plexus following surgical treatment of a lumbosacral myelomeningocele; the possibilities are discussed below. The last case of Winkelman and Moore [11] has a possible relation to a prolonged, difficult delivery requiring subsequent oxygen-carbon dioxide resuscitation.

Report of Cases

The following cases are presented as acute diffuse demyelinating encephalopathy of childhood, with certain unusual aspects.

Case 1

F. J. was a white female infant, delivered at term, with healthy parents and a normal, healthy brother of 5 years. There was no family history of nervous or mental diseases, and no congenital anomalies in the past four generations. This child was born with a lumbosacral myelomeningocele of 6 to 7 cm. in diameter and 2.5 cm. in height; a 2.5 cm. suboccipital meningocele; and widely separated suture lines and large fontanelles. The lumbosacral meningocele was tense, loculated, and translucent. Roentgenologic examination showed absence of the laminae and spinous processes from L.3 downward, including the sacrum. There was a defect of the skull which could allow a suboccipital meningocele. The skull appeared to show large loculi, which were interpreted as bony defects, a "Lückenschädel."

Laboratory findings were normal; Wassermann and Kahn tests were negative.

One month after birth the lumbosacral meningocele appeared larger and more

tense, and the fontanelles and sutures slightly more tense. The lower extremities were held in flexion and caused pain if extended, and there was complete skin anesthesia to pin-prick from L.2 downward. The levator ani and rectal and bladder sphincters were paralyzed.

Thirty-seven days after birth the lumbar meningocele was explored surgically and strengthened with lumbar fascia, under ether anesthesia; the dural sac was opened during the procedure, and nerve roots were seen floating in the fluid. Following operation there was progressive enlargement of the skull and there ensued mild fever ranging up to 99.5° F., which rose suddenly to 104° F. 1 week before death and continued to alternate between 103° and 106° F. until death. The child died at the age of 5 months, having reached a maximum weight of 13 lbs., 12 oz., with skull circumferences of 51.9 cm. suboccipitobregmatic, and 53.4 cm. occipitomental.

Clinical Diagnosis. Hydrocephalus.

Gross Findings at Autopsy

The body was that of a poorly nourished, under-developed infant showing tremendous enlargement of the skull, with broadly separated suture lines and large fontanelles. The lumbosacral scar showed no leakage. The various organs were essentially normal except for the central nervous system.

The brain weighed 1200 gm. It showed evidence of cerebellar herniation into the foramen magnum, and a distorted enlargement which had resulted from the differentially more rapid enlargement of the calvarium than of the base of the skull. The frontal lobes were therefore quite close to the brain stem, and the midcerebrum formed a great hump. The convolutions were broad, soft, often fluctuant, and so thin that on ordinary palpation the finger dropped through the surface into loculated cystic spaces containing clear, watery fluid. Sagittal sectioning revealed a swollen corpus callosum filled with cystic spaces containing clear fluid. Frontal sections showed a diffuse softening of the white matter throughout the hemispheres, with the tissue so edematous that its consistency was that of a very soft jelly. Isolated and loculated cysts of all sizes had formed. The process generally stopped abruptly at the cortex, although it sometimes also involved the inner cortical layers. Changes were most severe in the occipital region. The central gray masses were relatively free of this change. The ventricles were rather small and grouped close to the center of the brain, and all except the fourth ventricle were filled with semisolid, gruel-like, cheesy, white débris and had a firm zone of gliosis just outside the ependyma. A fibrosed mass of choroid plexus was seen in the roof of the fourth ventricle.

Hydromyelia was found in the upper half of the spinal cord, the central canal being irregularly dilated to 2 to 3 mm. Numerous tiny, fine, stringy adhesions connected the pia mater of the cord to the arachnoid, but the plane of cleavage was easily followed.

Histologic Findings

Throughout the white matter of the cerebral hemispheres and corpus callosum there was marked glial rarefaction, with only a few oligodendroglial and microglial cells and varying numbers of astrocytes present. These astrocytes were quite numerous in certain areas, notably near the basal ganglia, near some sections of cortex, and bordering cystic spaces. They tended to assume the gemästete form with a plump body of acidophilic cytoplasm and an eccentric nucleus. Clasmatodendrosis and other degenerative changes were seen only very infrequently, and then only in the most rarified areas. The cysts were found scattered throughout the white matter of both hemispheres and had replaced nearly all of the white matter in the left occipital lobe. Here they had interlacing trabeculae and walls of moderately dense glial tissue with numerous astrocytes. Several blood vessels here showed perivascular collars of lymphocytes and a few plasma cells; perivascular infiltration was insignificant elsewhere. An occasional scavenger cell contained droplets of neutral fat.

The cortex was relatively well preserved, except for some vacuolization and disarrangement of architecture in the occipital portion; and the basal ganglia, pons, and medulla showed no abnormality in sample sections. Demyelinization was confined to the white matter of the cerebral hemispheres and was almost complete, sparing only the arcuate fibers and an occasional islet. The axis cylinders, on the other hand, were destroyed only in the trabeculae and gliotic walls of cysts and near the ventricles.

Around the lateral and third ventricles there was a condensation of glia with a mixture of spongioblasts, astrocytes, and ependymoblasts with nuclei oriented more or less perpendicularly to the lumen. Islets and alveoli of ependymal cells were found only in the glial tissue around the third ventricle. Medial to the zone of gliosis, in all three ventricles, there was a lining of mesodermal granulation tissue, which in turn was lined by a mixture of compound granular cells, lymphocytes, and plasma cells. In the lumen there was a mass of fibrin and cellular detritus.

The choroid plexus of the third and fourth ventricles was partially buried in a mixture of glia and collagenous connective tissue, and the ependyma of the fourth ventricle was surrounded by a zone of gliosis and interrupted by numerous small areas of exuberant glial tissue protruding into the ventricle.

Around the pons and medulla there was fibrous thickening of meninges, and scattered groups of lymphocytes were present; elsewhere the meninges were normal.

In the spinal cord an intact layer of ependymal cells lined the dilated central canal, which had periodic (not segmental) outpocketings where the spinal cord became quite thin-walled. Some of the subependymal glia was arranged perpendicularly to the canal, but there was no increase in astrocytes. No parenchymal abnormality was seen. The pia mater was somewhat thickened and fibrous and had torn adhesions upon its surface.

Case 2

J. A. S. was a white male child, 1 year old, who had been well until the age of 9½ months, with normal birth and developmental history. He had had a mild upper respiratory infection for 1 week, was given a first injection of diphtheria toxoid, and had a severe generalized convulsion that same morning. A few hours later the temperature rose to 105° F., subsiding a few days afterward, and the patient then became comatose and remained so until death. He was first seen after the convulsion, when he was semicomatose, and was apparently blind and possibly deaf and displayed generalized spasticity with hyperactive reflexes.

The spinal fluid contained 7 lymphocytes per cmm., 64 mg. per cent protein, 741 mg. per cent sodium chloride, and 58 mg. per cent glucose. Blood and urine were normal. "Virus studies" were negative (type of study not stated).

Clinical Diagnosis. Encephalitis.

Gross Findings at Autopsy *

The head showed prominent frontal bossae and had the following measurements: suboccipitobregmatic circumference, 46.5 cm.; occipitofrontal, 45 cm.; biparietal diameter, 13 cm.; and bitemporal diameter, 10.5 cm.

The brain had edematous, flattened convolutions, was soft, and had an irregularly thin cortex which was accidentally pierced in a few places and immediaely poured forth clear, yellow fluid. After formalin fixation, frontal sections were made which showed a soft, spongy white matter with an extensive net of cavitation. The cortex was paper-thin in some areas. The central gray masses were somewhat soft but were without cavitation, and the cerebellum, midbrain, pons, and medulla appeared normal. There was moderate dilatation of the lateral and third ventricles and of the aqueduct of Sylvius. The other organs were essentially normal.

Histologic Findings

In the brain the basic change was a diffuse demyelinization and degeneration of the cerebral hemispheres, with involvement of the entire cerebral cortex and white matter, much of the basal ganglia on the left side, and a small area in the midbrain. In the left cerebral hemisphere the process appeared to have reached a termination with the formation of multiple cysts separated by glial trabeculae, while in the right hemi-

* Performed by Dr. William Brock.

sphere and the affected portions of gray matter in both sides the principal phase was that of glial proliferation.

In detail, the process appeared to start with an insidious diffuse increase of oligodendroglial and microglial cells (seen best in the right basal ganglia and in the pons), soon followed by disintegration of myelin sheaths and formation of numerous compound granular cells which took up the fat. This was quite marked in the right cerebral hemisphere. Edema separated the glial fibers, nerve cells and glial cells simultaneously degenerated, and perivascular infiltration of lymphocytes, plasma cells, a few neutrophils, and sometimes numerous compound granular cells occurred. When the degenerative process was not too rapid, there was an active proliferation of astrocytes, most of which were of the plump gemästete type, having a rounded, eosinophilic body, an eccentric nucleus, and either protoplasmic or fibrillary processes. Simultaneously with their proliferation, the astrocytes began to show degenerative changes such as clasmatodendrosis, extreme eccentricity or atrophy of the nucleus, and finally the production of a pink, oval body from the original cell. The process of degeneration may stop in the phase of gliosis and leave much of the tissue in that state, or it may pass through this stage or, if extremely rapid, never show gliosis at all but proceed directly to a final state of multiple cysts which are separated by thin trabeculae of glia and blood vessels.

Degenerating nerve cells sometimes became extensively ferruginated.

Comment

The first case is unusual in that the patient had developmental anomalies in the central nervous system; *i.e.*, a lumbosacral myelomeningocele, a suboccipital meningocele, and hydromyelia. Such patients usually develop hydrocephalus, but in this case the ventricles remained small. The diffuseness of the process in the brain, its limitation to subcortical tissue, the sparing of arcuate fibers, the relative severity of the occipital lesions, and the formation of cysts are all typical characteristics of acute diffuse subcortical demyelinating encephalopathy, or "encephalitis periaxialis diffusa."

In the second case the unusual feature is the nearly complete destruction of the cerebral cortex by continuation of the process. Cases of this type are well known and have been described by Alpers,[12] and others. This case presents the aspects of typical "sporadic, diffuse, cortical and subcortical demyelinating encephalopathy of infancy," according to Ferraro's nomenclature,[1] or "encephalitis periaxialis diffusa." The improbability of any direct relation of the disease to the administration of diphtheria toxoid has already been discussed.

Summary

1. Two cases of acute demyelinating encephalopathy of infancy, with some unusual aspects, are presented.

2. In view of the failure of either clinical or pathologic evidence to delimit completely and sharply any single group of cases of demyelinating encephalomyelopathy from all other such cases, it is believed advisable to unify all such diseases and then subdivide the group according to etiologic factors when such factors become known. Apparently "natural," clinical or pathologic, subdivisions, such as multiple sclerosis, should be retained only when such classifying aids in prognosis and in statistical search for etiologic factors, and should not be maintained as a list of discrete entities from which the clinician must "make a choice" in diagnosing the individual case.

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REGRESSION PRODUCED IN THE MURPHY LYMPHOSARCOMA BY THE INJECTION OF HETEROLOGOUS ANTIBODIES *

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Such a marked impression was made upon me by the destruction produced in normal mammalian tissues by the injection of heterologous anti-tissue sera that a way was sought to apply this technic to neoplastic tissues. When this was assayed three aspects of the problem immediately presented themselves: first, how to secure a sufficient quantity of antigen and prepare it in a relatively chemically pure state; second, the production of a potent antibody; and third, the determination of whether or not this antibody would have sufficient differential destructive action on the lymphosarcoma without harm to normal tissues or lethal effect upon the host. These problems were, in reality, those which had faced workers in the field of tumor immunity from its first inception. So much so, in fact, that although experimental work had advanced to the stage where it seemed likely that the antigen-antibody reaction was sufficiently specific to be utilized against neoplasia, so many intricacies were found that heterologous antibody studies were largely abandoned. There were those who frankly believed they had produced effective anti-tumor antibodies; others objected not only to the methods but also to the results. In time the work on tumor immunity has come to be presented largely by workers who have successfully prevented the growth of tumors in animals by injections of homologous tissues prior to tumor implantation. This is hardly a practical end and one which allows of little further experimental extension.

The first of these difficulties seemed approachable when Murphy ‡ and Sturm [1] produced a rapidly growing lymphosarcoma-leukemia in rats, a tumor from which, in a short time, with the use of a number of animals, as much as a kilogram of neoplastic tissue could be readily obtained. With a sufficient supply of antigen on hand it was then possible to do chemical fractionation upon the tumor, as well as to test it for antibody production in a number of foreign species. Further, dosage in these species could be determined; and, lastly, the rate of tumor growth was sufficiently constant, when these animals were inbred, to make it usable as one against which the antibodies might be tested. Supplementary studies were done in order to test for the presence of antibodies in the sera by *in vitro* methods.

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‡ Dr. James B. Murphy of the Rockefeller Institute for Medical Research kindly furnished the original tumor from which transplants were carried in Wistar (Carworth strain) rats.

Materials and Methods

Tumor Antigen Preparation

The Murphy lymphosarcoma was chosen for this work because of its rapid growth, high percentage of takes, and lack of necrosis. Those tumors which grew well and showed no gross necrosis were used in antigen preparation. They were chosen on the eighth to twelfth day after transplantation. After rapid dissection under aseptic conditions and within 5 minutes of the death of the animal the tumor was placed in thin slices in a sterile Petri dish set in solid carbon dioxide. The tumors thus harvested were then placed at —20° C. and held until 500 to 1,500 gm. were secured, ordinarily for 1 week. All antigens to be described were derived from this material.

The first tests were done with a whole tumor antigen prepared by grinding the frozen tumor. It was then extracted in cold physiological saline solution for 24 to 48 hours, centrifuged, and the supernatant liquid decanted. The supernatant liquid was used as one fraction. The residue was then washed several times with cold saline solution by centrifugation and the resulting solid portion resuspended in cold saline and used as a second fraction. These two antigens were injected intramuscularly into rabbits over a period of approximately 2 months at intervals of 5 to 6 days. The amounts injected ranged from 2 to 15 ml. in the case of the soluble antigen, and 1 to 8 ml. in the case of the insoluble antigen. On the tenth day following the final injection these rabbits were bled from the heart and the serum thus obtained was used.

Later chemical fractionation of the tumor was undertaken by two different methods and throughout the remainder of the experiments these fractions, together with whole minced tumor, were used as antigens. When the chemical fractions were given, the amounts were measured in milligrams. When whole minced tumor was used, the number of milliliters used was calculated to correspond to the number of milligrams, on the basis of the presence of 8 per cent solid protein in the tumor. Intravenous injections were found to be unsatisfactory and intramuscular injections were then resorted to exclusively. In relatively few instances these were found to cause sterile abscesses.

Tumor Antigen Injections

The chemical methods employed were derived chiefly from attempts to obtain different protein fractions. Thus one procedure was to prepare antigens by primary precipitation of the protein from a phosphate buffer extract of the frozen ground tumor, by acidification to pH 4.2 with acetic acid (method I). The abundant precipitate so obtained was taken off by centrifugation in the cold. The filtrate or supernatant

liquid was neutralized by ⅓ stepwise saturations with ammonium sulfate. A second procedure (method II) was straight stepwise saturation of the original material with ammonium sulfate at pH 7.0. These two methods with a classification of their precipitates are shown in Table I. Table II gives the nitrogen-phosphorus partition of the various frac-

TABLE I

The Two Methods of Chemical Treatment of Fresh-Frozen Lymphosarcoma (Murphy) Used to Obtain Antigens

Method I			Method II		
Fraction number	Method of preparation	Possible fraction	Fraction number	Method of preparation	Possible fraction
1. L. S. HAc	Ppt. from phosphate extract by acidif. to pH 4.2 with acetic acid	Nucleoprotein, any denatured protein	1. L. S. ⅓ sat., $(NH_4)_2SO_4$	Ppt. from ⅓ sat. of phosphate buffer extract	Nucleoprotein (?), pseudo- & euglobulins
2. L. S. ⅓ sat., $(NH_4)_2SO_4$	Ppt. from ⅓ sat. of neutralized filtrate from No. 1	Euglobulins, pseudoglobulins	2. L. S. ⅔ sat.	Ppt. by ⅔ sat. filtrate from No. 1	Globulins
3. L. S. ⅔ sat.	Ppt. from ⅔ sat. of filtrate from No. 2	Globulins	3. L. S. sat.	Ppt. by sat. filtrate from No. 2	Albumins, including contaminating serum albumins, and hemoglobin
4. L. S. sat.	Ppt. from sat. filtrate from No. 3	Albumins, including contaminating serum albumins, and hemoglobin			

TABLE II

Nitrogen-Phosphorus Partition of the Various Fractions Obtained in Methods I and II

Fraction number	Murphy Lymphosarcoma (rat)		
	N *mg. %*	P *mg. %*	P:N
HAc *I* from saline, no acetone treatment	13.9	1.5	1:9
HAc *I* from saline, treatment acetone, ether	14.6	1.44	1:10
Remainder of fractions not treated with acetone, ether			
Method I.			
1. HAc *I* from phosphate	11.0	1.78	1:6
2. ⅓ saturated	14.0	0.44	1:32
3. ⅔ saturated	16.0	0.099	1:161
4. Saturated	13.6	0.32	1:42
Method II.			
1. ⅓ saturated	9.44	1.50	1:6
2. ⅔ saturated	15.8	0.32	1:49
3. Saturated	13.6	0.50	1:27
Nucleic acid fraction from ⅓ saturated	11–12.6	6.70	1:1.64 1:1.88

tions. All precipitates were finally dried in the lyovac apparatus and injected into animals after resuspension in physiological saline.

Antigen Dosage

In order to ascertain the most effective dose an experiment was conducted in which the amounts of antigen given per injection were 1.0, 10.0, 50.0, and 100.0 mg. These were injected into rabbits at 3 and 4 days intervals for ten injections. Ten days following the last injection the rabbits were bled and the antibody titer determined by precipitin tests. Those rabbits receiving 1.0 and 10.0 mg. dosages were usually found to have a low titer of 1:256 or less, while in those receiving 50.0 and 100.0 mg. the titers tended to be higher, in the range of 1:1024. The day following this first bleeding, injections were begun again. Five of these were given, all of the rabbits receiving large doses, starting with 50 mg. and increasing to 250 mg. Ten days following this final injection they were bled again. In all instances they showed titers of 1:512 to 1:1024.

On the basis of the above experiment it was concluded that the large dosages were the more effective, and subsequent series of injections were conducted on that basis. A typical plan for antibody production follows:

Day of Injection	Amount of Injection
1st	50 mg.
3rd	100 mg.
6th	100 mg.
9th	100 mg.
13th	200 mg.
17th	200 mg.
22nd	200 mg.
27th	200 mg.
32nd	250 mg.
37th	250 mg.
47th to 50th	Bled

In addition to the rabbits employed, one series of ducks and two series of guinea-pigs were used. Equally satisfactory titers were obtained with the ducks, but since the rabbits were just as effective and far easier to care for, the ducks were not used. The guinea-pigs were found to be unsatisfactory, primarily because only low titers in the range of 1:64 could be obtained. Furthermore, it was impossible to obtain as large quantities of serum in return for comparable amounts of antigen administered.

The serum was obtained from the rabbits under aseptic conditions and after preliminary clotting was centrifuged from the clot as quickly as possible. It was then stored in sterile ampules until ready for use.

The sera were tested for precipitins only after each lot was tested on the animal which bore a tumor. The precipitin tests were set up by preparing the antigens according to their type.

Method of in Vitro Tests

For whole tumor antigen, fresh tumor was obtained and minced. A small portion of the minced tumor was then shaken thoroughly with cold normal saline and allowed to stand for at least 1 hour at 4° C.

The cloudy supernatant fluid was then withdrawn with a pipette and considered as undiluted antigen. The chemical fractions presented another problem inasmuch as they were largely insoluble. They were prepared ordinarily as 1 per cent solutions in normal saline, this concentration being taken as undiluted antigen. In some instances an attempt was made to put the more insoluble fractions into solution by adjusting the pH with 0.02 N. NaOH. In others they were prepared in 0.1 M. phosphate buffer solution rather than normal saline.

Since it is known that heat slowly inactivates precipitins, none of the sera were heated in these tests. The varying dilutions of antigen in 0.1 ml. amounts were layered onto 0.1 ml. amounts of serum in small precipitin tubes. In all instances suitable controls consisting of either saline or phosphate buffer solution, as determined by the preparation of the antigen, and of normal serum were included. The tubes were placed in a water bath at 37° C. for 1 hour. Sometimes they were examined for the presence of interfacial rings at the end of this period. This reading was never so high as that obtained after placing them in the ice box overnight. The preliminary reading was therefore discontinued and a single reading made following a minimum of 18 hours at a temperature of 4° C.

Method of in Vivo Tests

There was a certain percentage of spontaneous regressions with this lymphosarcoma (about 10 per cent, after considerable inbreeding of animals for tumor take and using just-weaned to 4 weeks post-weaned animals). For this reason care was exercised in the method of antibody testing. The plan was to test litter mates in equal numbers, half with control serum, half with antibody serum. The animals were inoculated from a fresh, viable tumor and the tumors given 4 to 5 days to establish themselves. After it was certain that the tumor was growing—they measured 12 to 18 mm. in longest diameter at this time—the animals were picked indiscriminately and numbered. The tumors were measured and after the animals were returned to their cage they were withdrawn at random and the first half then received the antiserum, and the second half, control serum. One observer made daily measurements without knowledge of the animals' numbers. A second identified the animals and recorded the measurements.

A preliminary series of six tumor-bearing rats received antiserum from fraction 1 of method I directly over the site of the tumor. All of these tumors regressed with heavy fibrosis about them. Six tumors, injected with control serum, grew without restraint. Since it could not be ascertained whether the fibrosis attendant upon the antibody injection was nonspecific, all subsequent injections were placed in a sub-

cutaneous region away from the tumor. The antiserum was administered in amounts ranging from 1 to 5 ml. at a single injection, and over a period of 10 days as much as 5 to 14 ml. was given.

There was amazingly little reaction from the antibodies derived from any of the antigens. In a large series of animals injected with rabbit serum containing antibody two died from what appeared to be sensitivity to the serum. Another reaction occurred—induration of the subcutaneous tissue at the site of antiserum inoculation. This was most common from fractions 3 and 4 antisera prepared by either method, and following massive doses into the subcutaneous region.

Results

The results are presented in three sections: (1) effects observed in tumor-bearing animals; (2) gross and microscopic observations on the treated animals; (3) observations in connection with *in vitro* precipitin tests.

Effects on Tumor-Bearing Animals

The percentage of regressions in the first series of experiments is shown in Table III. There was a 10 per cent regression in 83 animals which were not treated or which received normal rabbit serum. Seventy-

Table III

Summary of 10 Experiments Showing Results of Antibody Treatment of Rat Lymphosarcoma-Leukemia (Murphy); All Animals, Both Controls and Those Treated, Are Included

Test group	Serum titer and quantity	Total tumors	Number which grew at usual rate	Number whose growth rate was retarded	Number which regressed	Average interval before death in retarded group	Regression
Rats which received no serum	None	46	41	None	5	—	10%
Rats which received normal rabbit serum	None 7–14 ml.	37	33	None	4	—	10%
High titer antibody serum from antigen fraction 1	256–1024 8–14 ml.	22	3	7	12	19	54%
Low titer antibody serum from antigen fraction 1	64–128 5–10 ml.	28	7	17	4	25	14%
Antibody serum from antigens of all other tumor fractions	64–1024 7–14 ml.	25	16	4	5	40	20%

Regressions in all control animals	10%	83 animals
Regressions in all treated animals	28%	75 animals
Regressions in high-titer, high-dose animals	54%	22 animals

five animals treated with antibodies obtained in all fractions, and both minimal (5 ml.) and maximal (14 ml.) quantities of serum, gave 28 per cent complete regressions. Of these, the high-titer, high-dose animals, 22 in number, gave 54 per cent regressions.

It was discovered after completing the data in Table III that it was possible to correlate the percentage of regressions with the height of titer and dosage. If either the dosage or titer is low the tumor will not regress.

Fraction 1 antiserum made the most effective antibody.

TABLE IV

A Later Series of Experiments Than Those Shown in Table III

Antigen fractions	Antibody titer and quantity	Total tumors	Number which grew unchecked	Number retarded	Number which regressed	Average interval before death in retarded group	Regression
Normal rabbit serum (controls)	8–14 ml.	41	38	None	3	—	6%
Fraction 1. HAc	256–1024 8–14 ml.	21	2	7	12	19	57%
Fraction 1. HAc	64–128 5–10 ml.	23	11	5	7	28	30%
All other fractions	64–1024	25	16	4	5	40	20%

A later group of experiments, which gave almost identical results, is shown in Table IV. A somewhat higher average of total regressions, using all titers and dosages of antiserum, was obtained in this series.

The results, when the serum was used against animals previously inoculated with the tumor intraperitoneally (leukemic phase), are shown in Table V.

The growth curves (longest tumor diameter), of 15 nonregressing tumors treated with normal rabbit serum, are shown in Text-Figure 1. Text-Figure 2 shows an equal number of litter mate animals which bore tumors treated with anti-tumor serum. The difference in pattern is striking. Some animals which were treated with ineffective dosage and with sera of low antibody titer are included.

Gross and Microscopic Observations on Animals Treated with Anti-Lymphosarcoma Antibodies

Figure 1 shows a section taken from an animal which had received 12 ml. of normal rabbit serum. Those tumors, which were subsequently to regress under antibody treatment, showed softening within 24 hours following the first injection. The control tumors remained firm, pink, and extended in size. The regressing tumors became pale, and upon

palpation there was definite softening of the center of the tumor—evident necrosis. At 5 days to 1 week there was definite induration about the tumor, and if there was to be complete regression, there was a steady decrease in size. As may be observed from Text-Figure 2, all of the tumors did not regress. Even though it was not always possible to produce regression, often a great retardation in the rate of tumor progression would be brought about. On the average, death occurred later in all groups of treated animals with subcutaneous implants than in the controls. This was very notable in some groups, being particularly striking in those treated with antisera from all fractions other than 1.

TABLE V

Intraperitoneal Inoculations: Results of Antibody Treatment of Animals Inoculated Intraperitoneally with Rat Lymphosarcoma-Leukemia (Murphy)

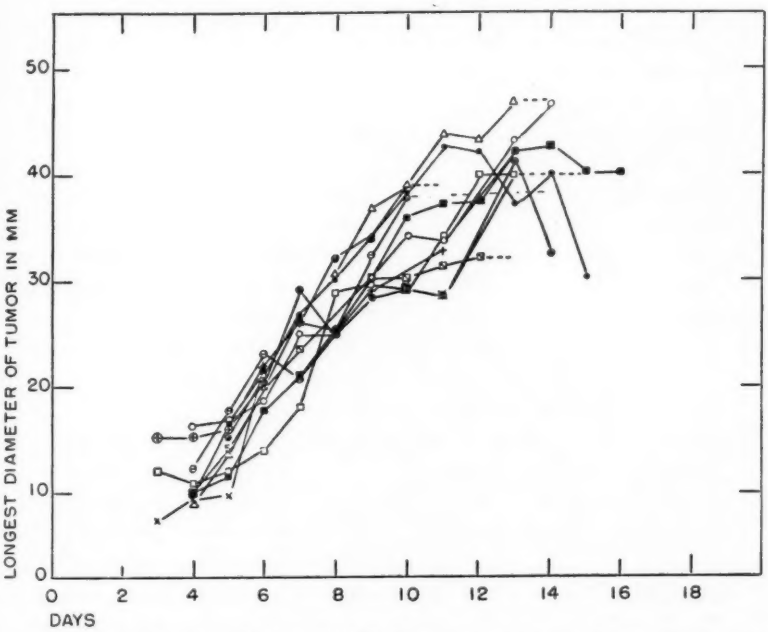
Test group	Serum titer and quantity	Total animals	Recovered	Retardation of clinical course	Number which died	Average interval before death
						days
Rats which received no serum	None	11	None	None	11	7
Rats which received normal rabbit serum	No titer 6–12 ml.	9	None	None	9	8
Antibody serum from antigen 1	Low and high 7–15 ml.	8	38%	——	5	8.5
Antibody serum from other fractions	Low and high 7–15 ml.	6	17%	1–25 days	5	9

In this group the average interval before death was 40 days, whereas the controls had died in an average of 13 days. One of these treated animals lived beyond the 50th day and very slowly developed an enormous tumor, which measured 70 by 55 by 20 mm.

Tumors which had been treated locally with the earlier and less effective antiserum showed viable lymphosarcoma cells heavily surrounded by a matrix of fibrous connective tissue (Figs. 2 and 3). This is quite similar to the picture seen late in other treated tumors, which, while they had not regressed, were greatly slowed in growth. These, also, showed scar tissue about them and a reduction in mitotic figures. Tumors which were successfully caused to regress with antiserum showed massive necrosis and fibrosis at the tumor site (Figs. 4, 5, and 6) with few to moderate numbers of giant cells. These two patterns were compared to those of a number of tumors which had spontaneously regressed (Figs. 7 and 8). There was no outstanding difference. The tumors which had spontaneously regressed tended to have less fibrous

connective tissue, more giant cells, and somewhat larger areas of calcification in the necrotic areas.

Examination of other tissues, with special attention to lymphoid tissue and bone marrow, showed no consistent differences in the organs of control, normal serum-treated, or antiserum-treated animals, except that almost all animals which had received maximal dosage of antibodies showed some hyperplasia of the spleen and bone marrow. There



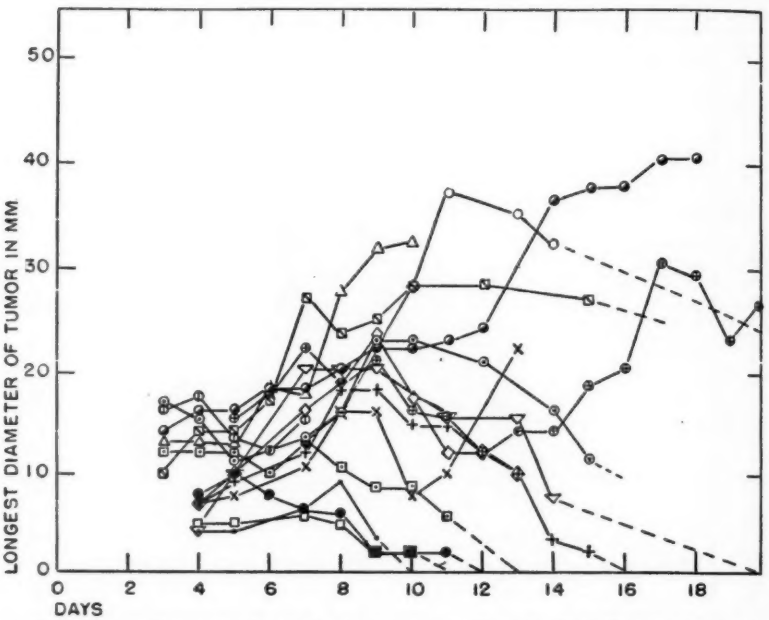
Text-Fig. 1. Curves made from the daily longest measurements of 15 tumors treated with normal serum.

were no late effects from this, since the tissues did not evidence necrosis or fibrosis. In areas of skin and subcutaneous tissue which had been removed from the indurated areas previously mentioned, there was considerable edema, mild inflammatory reactions, and some overgrowth of fibrous connective tissue.

Antigen-Antibody Reactions in Vitro

It is not possible to detail all of the results from the *in vitro* tests with the antigen-antibody reactions. Small 50 by 6 mm. tubes were used and dilutions of antigen from 1:2 through 1:1024 were obtained by doubling. After chilling in the ice box the tests were read in reflected

light; the precipitin was distinguished from the solid particles which tended to confuse the results. It was found that rabbits gave the highest consistent titers and the height of the titer depended upon a number of factors. Of these, the most important observable were: (1) the dosage, (2) length of inoculation period, and (3) the animal itself. Thus the highest titers were obtained in animals which received enorm-



Text-Fig. 2. Curves made in similar manner to those in Text-Figure 1 from 15 tumors treated with antisera.

ous doses of antigen over a long period, and of these animals some were high antibody producers, others were not.

Discussion

The present work encourages one to inquire further into the possibility of anti-tumor sera. There were deliberately sought, in this work, experimental conditions as nearly ideal as possible: abundant antigen, direct protein precipitation methods, massive doses of antigen over long periods in the heterologous species. In likelihood, the greater portion of antigen protein was derived from the lymphosarcoma nuclei, since they constituted the greater portion of the tumor mass. The antibodies against these proteins proved most potent in destroying the tumors. It is curious that the antibodies against constituents theo-

retically derived from the cytoplasm were most useful in prolonging the life of the animal when the tumor was not immediately destroyed.

It was discovered very early that here, as in bacterial diseases, potent antibody of sufficient quantity must be injected early to be of any value whatsoever. The injection of antibody serum in the late stages of the disease did not produce a single instance of regression. This suggests that, even in the face of some satisfying results, the antibodies prepared were not of great potency. Or if so, it must take large quantities to destroy effectively large numbers of individual tumor cells.

It was observed in those animals which bore spontaneously regressing tumors that the mechanism whereby lymphosarcoma cell destruction and subsequent fibrosis was produced came about with relentless certainty. The mechanism of this regression, if discoverable, should be of great value.

While it seemed possible to produce regression in transplanted lymphosarcoma with heterologous antiserum, it is not certain whether a similar result might be obtained in spontaneous tumors. These may differ markedly in their behavior from transplanted tumors. A statistically significant result was obtained in this work and it is hoped that the results soon will be tested against spontaneous tumors in animals.

Summary and Conclusions

1. By the use of rabbit anti-lymphosarcoma serum, it was possible to produce both retardation in rate of tumor growth and a significant reduction in mortality in rats bearing the Murphy lymphosarcoma.

2. Grossly, those tumors which regressed showed rapidly progressive necrosis, followed by fibrosis at the tumor site.

3. Microscopic examination of the tumor sites showed destruction of lymphosarcoma cells with massive fibrosis. This reaction did not differ markedly from that which occurred in spontaneously regressing tumors.

4. It was found necessary to utilize serum of high titer and in considerable quantity to effect those results.

5. The chemical fraction found most useful as an antigen was determined to be that which contains the nucleoproteins.

6. A prolonged immunization period, in rabbits, was needed to produce serum of high-antibody titer.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

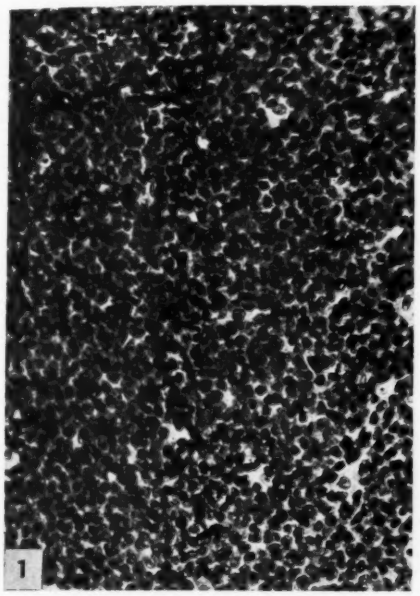
PLATE 89

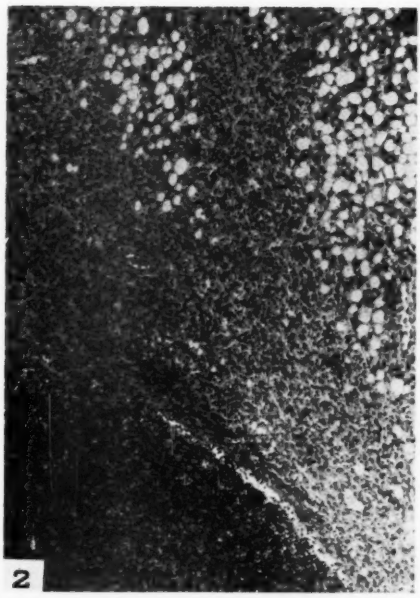
FIG. 1. Section removed on the eleventh day from a tumor in an animal which had received 12 ml. of normal (control) rabbit serum. The cell pattern is indistinguishable from that of tumors taken from animals which received no serum. × 250.

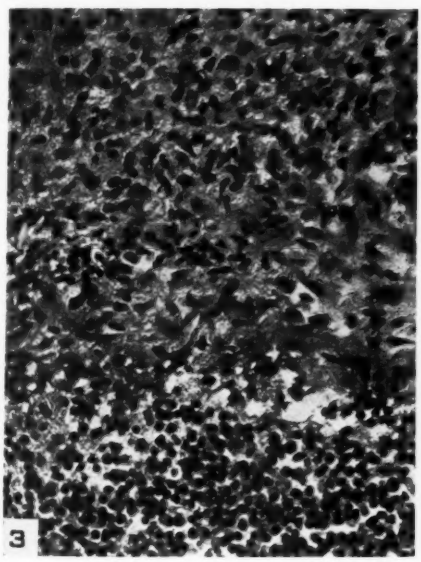
FIG. 2. This section of lymphosarcoma was removed from an animal which had received antiserum injections at the tumor site. Although there is abundant peri-tumor fibrosis, there is little necrosis of tumor cells. × 70.

FIG. 3. Higher-power field from the same tumor as shown in Figure 2. The lymphosarcoma cells are below. ×250.

FIG. 4. Section from tumor site in an animal which had received 14 ml. of high-titer antiserum (fraction 1, method I) subcutaneously and not at the tumor site. × 70.







Nettleship

Regression in Lymphosarcoma

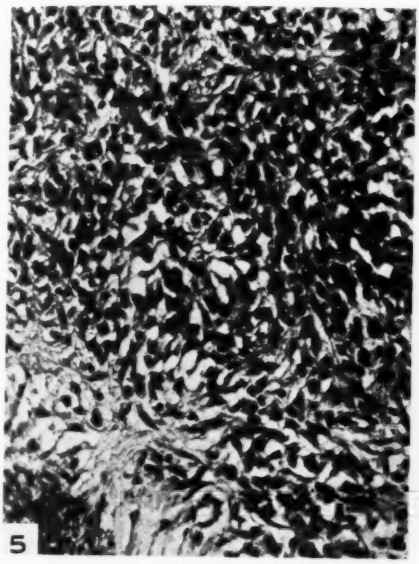
PLATE 90

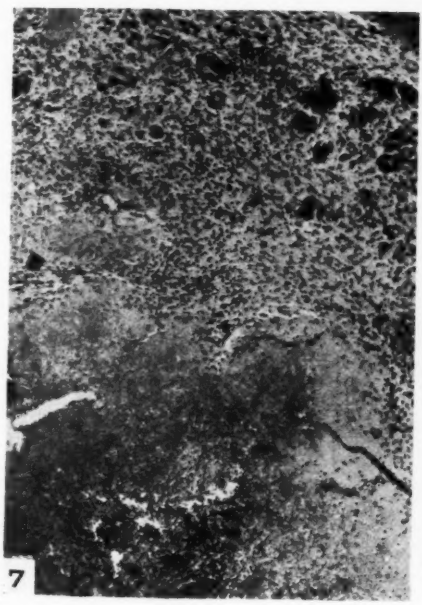
FIG. 5. Taken from same section as Figure 4. Only fibroblasts and an area of necrosis (in the lower left corner) are seen. × 250.

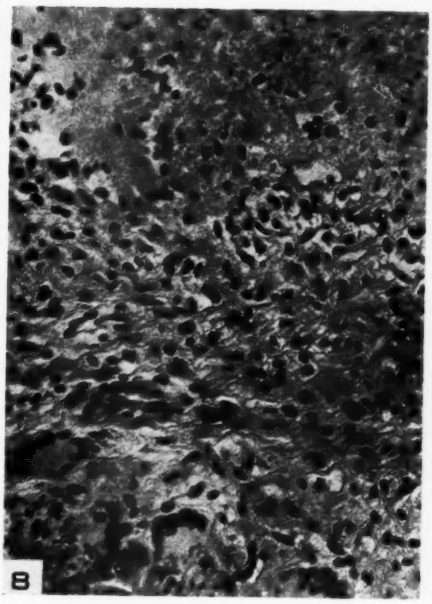
FIG. 6. Another tumor site from a high-titer, antiserum treated animal, taken on the tenth day. × 250.

FIG. 7. Low-power field of a section of tumor which had spontaneously regressed, taken on the 13th day. There is fibrosis and necrosis. The granular black areas are areas of calcification. × 70.

FIG. 8. From the same section as Figure 7, showing giant cells in the lower portion of the field. × 250.







Nettleship

Regression in Lymphosarcoma

THE DIAGNOSIS OF GRANULOMA VENEREUM FROM FROZEN SECTIONS STAINED WITH POLYCHROME METHYLENE BLUE *

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Since Donovan's original observation [1] of the constant presence of specific intracellular inclusions in the lesions of granuloma venereum, the use of tissue smears stained by variations of the Romanowsky method has been a standard laboratory procedure in the diagnosis of this disease. But the indifferent staining of these bodies with hematoxylin and eosin, their failure to retain the Gram stain, and the variable results obtained by modifications of the Romanowsky stain long discouraged the use of histopathologic methods for the study of this disease. Practical and reliable technics for the demonstration of these organisms in sections were not reported until 1937, when Pund and Greenblatt, using Delafield's hematoxylin [2] and eosin, and Dieterle's silver impregnation method,[3] were able to describe the specific character of the intracellular parasitism of the disease.[4, 5]

To augment the simple, rapid, smear technic, and the section methods of Pund and Greenblatt as laboratory procedures in the study of granuloma venereum, one further observation is recorded here. In frozen tissue sections, stained with Terry's polychrome methylene blue, the histologic features of the disease are brilliantly demonstrated.

Terry's neutralized polychrome methylene blue [6, 7] is made up as follows:

1. Stock solutions, in neutral distilled water:

   A. 12% anhydrous potassium carbonate, C.P..... 100 cc.
   B. 1% methylene blue (Grübler's or Merck's medicinal) . 1000 cc.
   C. 10% acetic acid, by volume..... 100 cc.

2. Titration. Dilute 1 cc. of solution C with 15 cc. of distilled water. Add as an indicator 5 drops of phenolphthalein. Heat to boiling and determine how much of solution A is required to neutralize. Mark this quantity on bottle A.

3. Alkalinization. Into a 100 cc. graduate, place that quantity of A which is equivalent to 1 cc. of C, add enough of B to make 100 cc. and mix thoroughly.

4. "Polychroming." Of the alkalinized methylene blue, 25 cc. are placed in each of four 1 oz. bottles. These are stood unstoppered in cold water. This is brought to a boil in about 10 minutes and kept boiling during the procedure. Remove the bottles, one by one, 15, 20, 25, and 30 minutes after boiling has begun.

5. Neutralization. To each 25 cc. of the polychromed stain add 0.25 cc. of solution C.

Filtration is usually unnecessary and should not be carried out for 1 or 2 days. The four different bottles contain stain of different polychromatic values, the richest one being that which was allowed to digest longest. Any of the four will be satisfactory. Generally speaking, the richer the stain in colors, the better the

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results with unfixed tissues. Tissues either fixed rapidly in heated formalin, or fixed routinely in formalin, stain readily with the solutions poorer in color. The neutralized stains are stable.

For the type of tissue usually obtained from lesions clinically resembling granuloma venereum, formalin fixation, either rapid or routine, is recommended, although unfixed tissues may be used if desired. Frozen sections, cut at 20 to 25 μ, are floated or dipped in the dye for a few seconds, the time required for staining being determined by trial. The sections are then washed and mounted on a slide in water. From time to time a drop of water may be added to the edge of the coverslip to keep the preparations wet. Tissues stained by this method cannot be satisfactorily preserved, but they may be kept long enough to allow careful study.

A detailed description of the frozen section technic is to be found in the article by Forbus.[7]

The appearance of the intracellular parasites in these preparations is essentially similar to that obtained with other methods, but certain differences are apparent. The flatness of contour and the cellular disruption seen in smears are eliminated, and the shrinkage and possible distortions resulting from dehydration, clearing and mounting in the other section methods are avoided. Large parasitized mononuclear cells, 20 μ or more in diameter, are readily recognized under the high-dry objective, and they can be given detailed study under oil immersion. The clear cystic cytoplasm of the invaded cells often makes them conspicuous even in relatively thick preparations (Fig. 1). A peripheral distribution of bodies within the intracytoplasmic cysts is sometimes observed (Fig. 2) but, perhaps because of the wide range of focus possible, this feature does not have the prominence observed in paraffin sections. In such cells a slight alteration of the focal plane will obliterate the "ringed" pattern of the bodies (Fig. 3). Some intracytoplasmic cysts are completely filled with the organisms (Figs. 4 and 5). Less heavily infected cells may contain no cysts. Although parasitism is essentially a feature of mononuclear cells, occasionally single bodies can be seen in polymorphonuclear cells (Fig. 5). Extracellular bodies can be recognized singly and in small groups.

The Donovan bodies are stained a delicate blue. The staining is diffuse, with no strong tendency toward polar staining, and no metachromatic granule or nucleus can be demonstrated within the body. The peripheral zone, or capsule, is clear and unstained (Figs. 4 to 7). The bodies have a variable coccobacillary shape, and occasionally diplococcal forms are observed within a continuous capsular membrane (Fig. 7), indicating a process of fission. The organisms usually

measure 0.6 to 1.8 μ in length, and 0.6 to 0.8 μ in diameter, with the investing capsular zone being about 0.3 μ in thickness. Extremely elongated bacillary forms have not yet been seen with this technic.

The granules of mast cells are stained a reddish purple in these preparations, and cannot be confused with the organisms. The Donovan bodies appear, however, so similar to other bacterial forms that the diagnosis of the disease cannot be made on the basis of their morphology and staining characteristics alone. The characteristic parasitism of mononuclear cells must be observed. Although this feature of the disease is strikingly similar to that observed in leishmaniasis and histoplasmosis, the smaller size of the Donovan bodies enables them to be differentiated from the other agents on a morphological basis alone.

If granuloma venereum always presented a straightforward clinical picture, the smear technic and the slower section methods of Pund and Greenblatt [4] would be adequate procedures for the diagnosis and study of the disease. But the tendency of this condition to simulate other processes, especially in the cervix uteri where the lesions are frequently diagnosed as carcinoma,[5] makes it desirable to have available a rapid and efficient biopsy technic. This need is satisfied by the frozen section method. This compares in effectiveness with the standard procedures and, for laboratories serving outside hospitals and receiving fixed materials for study, it is the only rapid method available. It has the disadvantage that the preparations cannot be satisfactorily preserved, so that it has to be supplemented by the longer section methods in order to obtain permanent records. So little tissue need be used in the procedure that the usual specimen removed for biopsy will serve for both procedures. For photomicrographic study and camera lucida drawings, carefully prepared sections are superior to any other preparations.

Summary

The use of frozen sections stained by Terry's polychrome methylene blue is recommended as an aid in the diagnosis and study of granuloma venereum. The technic satisfies the need for a rapid and efficient method suitable for all types of tissues.

The details in the morphology of the Donovan bodies, especially the capsular zone, are demonstrated better by this technic than by any of the other section methods. These features are briefly described.

The thickness of the sections allows a wide range of focus, and it is often possible to obtain a three dimensional study of the parasitized mononuclear cells. This affords a particularly good opportunity for the study of the peculiar intracytoplasmic distribution of the bodies. A

comparison is made of the pattern observed in frozen sections with that seen in sections prepared by other methods.

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DESCRIPTION OF PLATE

PLATE 91

The photomicrographs, using Wratten filters B and K1, are of frozen sections stained with Terry's neutralized polychrome methylene blue.

FIG. 1. Parasitized cell. The cystic cytoplasm stands out against the dark background of the thick preparation. × 1275.

FIG. 2. Parasitized cell, showing peripheral distribution of Donovan bodies within intracytoplasmic cysts. × 1235.

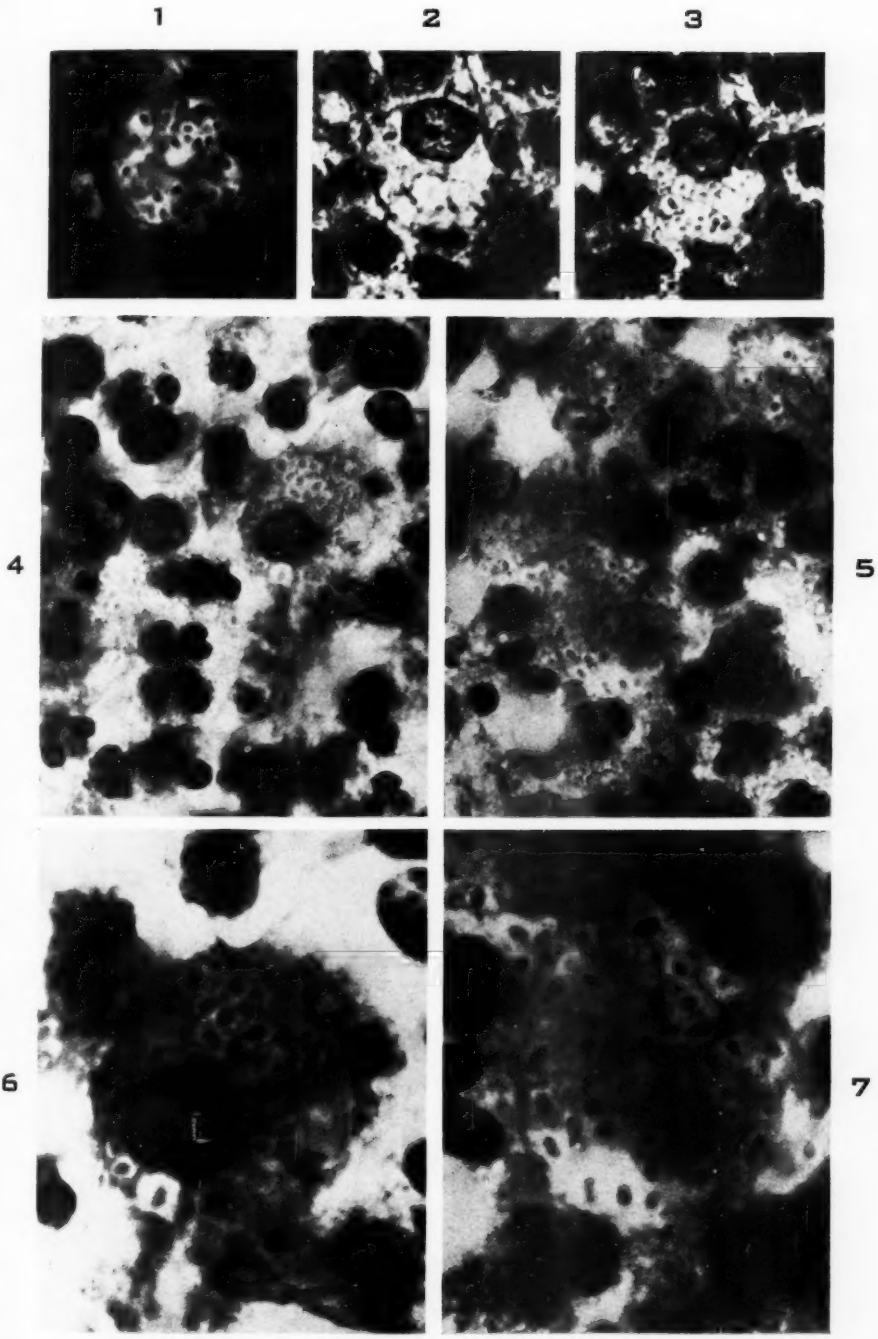
FIG. 3. The same cell, at a slightly altered focus. The ringed pattern of the bodies has disappeared. × 1235.

FIG. 4. The character of the inflammatory exudate and the mononuclear parasitism are shown. × 1275.

FIG. 5. Several parasitized mononuclear cells are included. Below the large central mononuclear cell a polymorphonuclear cell is seen containing a single body. × 1275.

FIG. 6. A portion of Figure 4 enlarged to show details of the morphology of the Donovan bodies. × 2550.

FIG. 7. A portion of Figure 5 enlarged to illustrate details in the structure of the Donovan bodies. The pleomorphism of the organisms, the capsular zone, and diplococcal forms are demonstrated. ×2550.



Margolis Diagnosis of Granuloma Venereum

ARTERIAL OCCLUSIONS PRODUCED BY EMBOLI FROM ERODED AORTIC ATHEROMATOUS PLAQUES *

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In a recent autopsy of a man with advanced arteriosclerosis, changes were observed in some of the small and medium-sized arteries of the kidney, spleen, pancreas, and thyroid. In the lumina of these vessels were spaces with the shape of cholesterol crystals and a few slit-like endothelium-lined channels. Foreign body giant cells partly surrounded many of the cholesterol crystal spaces. These occluding lesions were found in arteries having an external diameter of from 55 to 900 μ. Their appearance suggested that emboli, containing large cholesterol crystals, had lodged in these vessels and undergone organization. Since there was advanced erosion of the atheromatous plaques in the aorta of this man, it was believed that these eroded plaques were the source of the emboli.

It is known that the contents of an atheromatous plaque may serve as emboli if the surface of the plaque undergoes erosion,† [1, 2] and occlusion of coronary arteries by similar emboli has been described.[3]

Since there have been no recent studies on this subject, this investigation was made.

MATERIAL AND METHODS

Two hundred and sixty-seven autopsies were selected for review. Of these, 233 had been diagnosed as having "advanced arteriosclerosis" in the aorta. All of these had many atheromatous plaques in the intima of the aorta, and in some instances the atheromatous areas had eroded and were partially covered with mural thrombi. Thirty-four other cases were reviewed because the description of the aortas suggested that the degree of atherosclerosis was actually "advanced" although in the anatomic diagnoses it was called "moderate."

Of the 267 subjects, 191 were males ranging in age from 33 to 92 years, and 76 were females of from 35 to 86 years. One hundred and forty males and 57 females were over 60 years of age. The average age of all patients was 64.9 years. Sections of the kidney were ex-

* Received for publication, June 29, 1944.

† The term "erosion" is used in this paper to describe the process of breaking down of the intimal surfaces of atheromatous plaques. This is seen commonly in the aortas, and occasionally in other arteries as well, of people with advanced arteriosclerosis. Since the flowing stream of blood is the force which removes the material of the plaque, the term "erosion" is appropriate in both geologic and pathologic senses. It is unfortunate that the term "ulceration" is used in some texts to describe this process, as the lesions produced are not inflammatory in origin and have no etiologic or histologic resemblance to ulcers.

amined in 244 cases, of the pancreas in 256 cases, of the spleen in 251 cases, of the adrenal in 109 cases, of the thyroid in 16 cases, and of the prostate in 20 cases. Often more than one section of an organ was examined. In selected cases serial sections were cut and the vascular changes studied in detail.

Vascular occlusions were found in 9 of these autopsies, yielding an incidence of 3.4 per cent among the 267 patients with advanced arteriosclerosis of the aorta.

Most of the sections were stained with hematoxylin and eosin; certain of them were stained also with Weigert's stain for elastic fibers. Frozen sections were cut of formalin-fixed tissues from case 1, and stained for fat with Sudan III.

The dimension of an artery was obtained by measuring the external diameter of the media with a calibrated ocular micrometer. This was done in the sections of fixed, paraffin-imbedded material.

The term cholesterol crystal spaces is used to describe the slit-like spaces which remain in tissues at the sites of cholesterol crystals dissolved during the preparation of the sections.

Report of Cases

Case 1 is given in detail. In Table I are listed the age, cause of death, blood pressure, and the degree of arteriosclerosis found at autopsy in each of the 9 cases. The distribution and frequency of the arterial occlusions are summarized in Table II.

Case 1

A white male, 61 years old, had a blood pressure of 180/100. He died with symptoms of coronary thrombosis.

At autopsy (autopsy no. 11016; by Dr. C. M. Flory) the entire aorta was markedly arteriosclerotic. In even the ascending portion and arch of the aorta some atheromatous plaques were superficially eroded and covered with mural thrombi. In the remainder of the aorta the intima was a mass of confluent atheroma, most of which was eroded, and covered with thrombi. Calcification was scanty. The coronary arteries contained many large atheromatous plaques and in places their lumina were stenosed. The heart weighed 710 gm.; its left ventricle was greatly enlarged. Healed infarcts were present in the myocardium. The iliac, splenic, celiac, right renal, and other medium-sized arteries were dilated and tortuous. The left kidney was a fluid-filled sac, and its ureter occluded by a scar. The right kidney weighed 155 gm.; its cortical surface was indented by deep wedge-shaped scars. In the area between the scars the cortex was of normal thickness. The small renal arteries were thick-walled. The spleen, pancreas, and thyroid gland appeared normal.

Histologic Observations

Great Vessels. In the intima of the aorta, 1 cm. above the aortic valve, were thick atheromatous plaques filled with cholesterol crystal spaces and lipid-filled macrophages. In the lower portions of the aorta the intima was 2.5 mm. thick and filled with structureless, gruel-like material containing many cholesterol crystal spaces. In some areas the intimal lining was eroded, and mural thrombi containing crystal-shaped spaces were attached to the atheromatous material (Fig. 1). About some of the cholesterol crystal spaces were foreign body giant cells.

Right Kidney. The cortex of the right kidney was slightly thinner than normal, and beneath the wedge-shaped depressed areas the glomeruli and tubules were atrophic or hyalinized. The large and small renal arteries were thick-walled. Their intimas were greatly thickened, and there was much reduplication of the internal elastic lamina. The arterioles were hyalinized.

TABLE I

Clinical and Pathologic Data on Nine Cases with Emboli from Atheromatous Plaques

Case number*	Autopsy number	Age	Cause of death	Blood pressure	Arterio-sclerosis of aorta	Erosion of atherosclerotic plaques in aorta			Mural thrombi in aorta
						Thoracic aorta	Abdominal aorta	Site not stated	
1	11016	61	Coronary thrombosis	180/100	+++	++	+++		+++
2	8526	70	Cellulitis of neck	184/94	+++	0	++		0
3	9146	58	Coronary thrombosis	?	+++			+++	++
4	9855	69	Pyelo-nephritis	240/120	+++			++	++
5	10204	48	Carcinoma of stomach	140/80	+++	0	+++		++
6	10453	72	Coronary thrombosis	170/120	+++			+++	?
7	10633	67	Broncho-pneumonia	175/100	+++	+++	+++		0
8	10644	69	Peritonitis after lapa-rotomy	130/70	+++			+++	+
9	11226	50	Coronary thrombosis	160/108	+++	+++	+++		++

* All patients were males.
0 = No lesion.
+ = Slight changes present.
++ = Moderate changes present.
+++ = Advanced changes present.
? = Information not given.

Cholesterol crystal spaces, partly surrounded by giant cells, and a few slit-like vascular spaces were seen in the lumina of the arteries in each of the 5 blocks of tissue examined. In 174 arteries over 50 μ in external diameter, 20, or 11 per cent of the total, contained these lesions. The arteries involved varied in diameter from 58 to 880 μ.

In a typical lesion the intima of the artery was hyperplastic and the entire lumen was filled with loose connective tissue surrounding choles-

TABLE II

Distribution and Frequency of Arterial Occlusions

Case number	Heart	Lung	Liver	Spleen	Pancreas	Kidney	Adrenal	Thyroid
1	o	o	o	++	+	++	o	+
2	o	o	o	+	+	+	—	o
3	o	o	o	+	+	+	—	—
4	o	o	o	o	+	o	o	—
5	o	o	o	o	o	+	—	—
6	o	o	o	+	+	o	—	o
7	o	o	o	o	+	o	—	—
8	o	o	o	+	o	o	—	—
9	o	o	o	+	+	o	—	o
Totals	o	o	o	6	7	4	o	1

0 = Organ contained no occluded arteries.
+ = Organ contained one or few occluded arteries.
++ = Over 10% of arteries in the organ were occluded.
— = Organ not examined histologically.

terol crystal spaces and a few thin vascular channels. The crystal-shaped spaces were frequently bordered by foreign body giant cells.

A large lesion is shown in Figure 2. This was in an artery measuring 880 μ in diameter. In its lumen were four large cholesterol crystal spaces, about two of which were foreign body giant cells. Several small vascular channels ran through the hyperplastic intima which surrounded the crystal spaces. Many hemosiderin-filled macrophages were present, suggesting either that this was an organizing embolus or that there had been hemorrhage into this lesion. On following this lesion in serial sections, the pattern shifted rapidly, but the component parts—vascular channels, cholesterol crystal spaces, and giant cells—remained. This occluded vessel lay in the base of a large wedge-shaped area of cortical atrophy.

Another occluded vessel is shown in Figure 3. This measured 825 μ in diameter and was filled with small vascular channels and by large

cholesterol crystal spaces partly surrounded by giant cells. A few of these crystal-shaped spaces contained a homogeneous pink-staining, protein-like material, apparently the matrix of the crystals.

An earlier lesion is shown in Figure 4. A large branch of an artery measuring 920 μ across was plugged by cholesterol crystal spaces. These were partly surrounded by giant cells and loosely attached to the wall by hyperplastic intima. The mass projected into the lumen of the smaller vessel from the larger one.

Another type of lesion, consisting largely of vascular channels, is shown in Figure 5, A to E. This occlusion began at the bifurcation of an artery with diameter of about 600 μ. The first portion of the lesion consisted of a V-shaped group of vascular channels surrounding a few cholesterol slits (Figure 5-A). The bifurcation of this vessel is seen in Figure 5-B and its two channels in 5-C. In Figure 5-D the branches are separated and the lower branch is filled with cholesterol crystal spaces. These vessels lay in the base of a wedge-shaped area of cortical atrophy (Fig. 5-E). Many small branches of these arteries were filled similarly with crystal-shaped spaces. No hemosiderin was seen.

Many smaller vessels in the kidneys were also involved. These arteries varied from about 60 to 200 μ in diameter. In some the occlusions were similar to those in larger arteries. In others (see Figs. 8 and 9 of small splenic arteries) the cholesterol crystal spaces lay in the innermost portion of the thickened intima, and the vascular channel passed to one side. In some sections macrophages with vacuolated cytoplasm lay near the crystal spaces, which were partly surrounded by giant cells.

Frozen sections were cut from formalin-fixed blocks of the kidney tissue, stained for fat with Sudan III and counterstained with hematoxylin. Most of the cholesterol crystals did not remain *in situ* even in the frozen sections. In a few vessels, however, thin, rectangular crystals were seen. Most of the lipid which stained with Sudan III was in the outer portions of the intimas of the occluded vessels. The cholesterol crystal spaces were always found in the former lumina of the vessels, and often were surrounded by lipid-free tissue. In other vessels some lipid was present about the cholesterol crystal spaces, but the amount was never so great as in the outer layers of the intima.

Left Kidney. The glomeruli and tubules of the left kidney were atrophic and fibrosed, and the cortex and medulla were very thin. The arteries had thick walls but contained no lesions.

Spleen. The splenic pulp and malpighian bodies were normal. In the small arteries the intima was hyperplastic, and reduplication of the internal elastic lamina was prominent. The arterioles were hyalinized.

Two blocks of the spleen were available for study. In single sections

of both blocks were 63 arteries with a diameter of 50 μ or over, of which 12, or 19 per cent, had these lesions in their lumina. The occluded vessels varied from 58 to 540 μ in diameter. Three occluded splenic arteries (the largest measuring 540 μ) are shown in Figure 6. In branches of these arteries also the lumina were filled with cholesterol crystal spaces.

Figure 7 is a small branch of a larger occluded artery; the foreign body giant cells about the crystal spaces are clearly shown. Lesions of different histologic appearance than that seen in Figure 7 are shown in Figures 8 and 9. The vessel illustrated in Figure 8 measured 84 μ in diameter. Its muscularis and adventitia were vacuolated and contained scattered lymphocytes. Cholesterol crystal spaces, partly surrounded by giant cells, lay against the intimal surface of one side of the artery. In the second vessel (Fig. 9) the crystal-shaped spaces were attached to the surface of the intima and were surrounded by giant cells. A large vascular channel remained.

Pancreas. Histologic examination of the pancreas showed the parenchymal cells and islets to be normal. Fibrous tissue was excessive between the lobules of the gland. Intimal hyperplasia was marked in the medium-sized and small arteries. The arterioles were hyalinized.

Four arteries, varying in size from 77 to 270 μ in diameter, contained these lesions. The lumen of the largest was filled with intimal tissue in which were several slit-like vascular channels and two cholesterol crystal spaces surrounded by giant cells. The lesions in the other arteries were similar. One segment of a small artery contained a typical lesion; the next segment of the vessel was almost obliterated by intimal hyperplasia and by an accumulation of macrophages with lipid-filled cytoplasm.

Thyroid. The small arteries of the thyroid gland were thick-walled and their intimas hyperplastic. In the lumina of two small arteries were cholesterol crystal spaces and giant cells.

Other Organs. No occluding vascular lesions were found in the heart, lungs, liver, or adrenals.

Relation of Arterial Occlusions to Arteriosclerosis and Other Diseases

The 267 cases can be divided into three groups. In 63 cases erosion of atherosclerotic plaques in the aorta was not noted. No arterial occlusions containing cholesterol crystals were found in these cases. The second group consisted of 147 cases in which the erosion was of slight or moderate degree. Only two instances of these arterial occlusions (cases 2 and 4) were found in the group, an incidence of 1.3 per cent. In the third group the erosion of the plaques in the aorta was marked. Such advanced erosion was found in 57 cases, of which 7, or 12.3 per

cent, had arterial occlusions containing cholesterol crystals. The absence of this lesion in patients without advanced arteriosclerosis of the aorta has been substantiated by studying our routine autopsies. No arterial occlusions containing cholesterol crystals have been found in over 200 of these patients.

The occlusions have not been found among the very old, but in patients from 48 to 72 years of age, with an average age of 62.5 years. All were males. Of the 9 patients with this lesion, 7 had hypertension, 8 had narrowing or occlusion of coronary arteries, and 5 had myocardial infarcts. No patient, however, had diabetes, and only 2 had positive serological tests for syphilis.

Origin and Development of the Arterial Occlusions

The only early lesion found was a thrombus containing large cholesterol crystal spaces. This was attached to the wall of a medium-sized artery in the kidney of case 5 (Fig. 12). It seemed almost certain that these crystals were dislodged from eroded atheromatous plaques in the aorta and carried as emboli into this vessel.

The mass of cholesterol crystal slits projecting into the lumen of a medium-sized vessel of case 1 (Fig. 4) was an older, better organized lesion. There was a moderate degree of intimal proliferation about these crystal-shaped spaces. No remnant of the thrombus remained. Although many other medium-sized arteries were occluded in case 1, no crystal-shaped spaces were found within recent thrombi. The presence of many hemosiderin-filled macrophages in one occluded vessel (Fig. 2) suggested that a thrombus might have been present and organized.

Fully developed, well organized lesions were found in the medium-sized arteries in cases 1, 2, 3, and 9. The lumina of these vessels were filled with cholesterol crystal spaces surrounded by a few foreign body giant cells and intimal tissue. This type of occlusion is shown in Figures 2, 3, 5, and 6.

In every case except case 5 some small arteries measuring from 50 to 200 μ in diameter (Figs. 7 to 11) were partially or completely occluded by these lesions. No arterioles were affected. The histologic appearance of these occlusions in small vessels was more variable than that in the medium-sized vessels. In some of the small arteries the lumina were plugged by masses of cholesterol crystal spaces partly surrounded by giant cells. Figures 7 and 10 illustrate such lesions. Many of the small occluded arteries in case 1 and other cases were actually branches of larger obstructed vessels.

Emboli of cholesterol crystals from eroded aortic atheromata seemed to explain the origin of all of these lesions satisfactorily. In some small arteries, however, the cholesterol crystals lay against the hyperplastic

intimas of vessels in which the vascular channel was not occluded (Figs. 8, 9, and 11). It seemed possible that such cholesterol crystals might have been formed from the lipids in the thickened intima of the vessels. They probably, however, were examples of advanced recanalization of a vessel previously occluded by cholesterol crystal emboli.

It is believed that the arterial occlusions containing cholesterol crystal spaces are the result of organization of emboli from eroded aortic atheromata. The mass of cholesterol crystals, mixed with lipid and thrombus material, is torn loose by the flow of blood and is carried into a medium-sized or small artery, where it lodges. About this embolus a thrombus forms and organizes. The blood clot is removed, but the cholesterol crystals remain and are encased by intimal tissue and foreign body giant cells. Recanalization of the thrombus takes place between or beside the crystals, forming slit-like vascular spaces. In a completely organized lesion the artery is occluded by cholesterol crystals, often surrounded by foreign body giant cells, slit-like vascular spaces, and hyperplastic intimal tissue.

The only anatomic changes associated with these arterial occlusions were found in the kidney where the renal parenchyma supplied by these obstructed vessels was atrophic, forming depressed, wedge-shaped cortical areas (Fig. 5-E). No changes attributable to these vascular lesions were observed in the pancreas, spleen, or thyroid.

Experimental Production of the Lesions

An unfixed human aorta was selected in which atherosclerosis was marked. Soft yellow material was scraped from several of the plaques and suspended in 5 cc. of physiologic saline solution. Microscopic examination of this fluid revealed many large, clear, thin rhomboidal crystals having the characteristic shape of cholesterol crystals. Fat droplets and red blood cells also were present.

Two and one-half cc. of this material was injected into the ear veins of 2 rabbits. One animal was killed after 24 hours. In its lungs many small arteries were occluded by masses of red blood cells, polymorphonuclear leukocytes, and large cholesterol crystals. The other animal was killed after 7 days. Many small pulmonary arteries were also occluded. In these vessels the cholesterol crystals were no longer surrounded by leukocytes but by foreign body giant cells and hyperplastic intimal tissue (Figure 13).

Discussion

The embolic theory of the origin of these arterial occlusions has been discussed. Another explanation is that the crystals formed *in situ* in the hyperplastic, lipid-rich intima of the arteriosclerotic vessels and

that the entire process is an unusual form of arteriosclerosis. If the interpretation of the histologic appearance of these lesions presented previously is correct, the arteriosclerotic hypothesis seems untenable.

Additional evidence against the theory of the formation of the crystals *in situ* in the arteries is the fact that in 63 patients with advanced arteriosclerosis of the aorta but with no erosion of aortic atheromatous plaques, crystal-containing arterial lesions were not found despite the fact that the splenic, pancreatic, and renal arteries were often very thick-walled and contained much lipid in their hyperplastic intimal tissues. In the 147 patients with slight or moderate erosion, the lesion was found in only 1.3 per cent. However, of the 57 cases with advanced erosion of atheromatous plaques in the aorta, 7, or 12.3 per cent, had these lesions. This suggests that embolism rather than arteriosclerosis is the cause. It may be argued, however, that the arteriosclerosis was actually more marked in the latter groups. This certainly was true in the aorta, but there was no histologic evidence that the arteriosclerosis in the smaller vessels was more severe in one group than in the others.

Another argument against the theory of formation of these crystals *in situ* is the location of the crystals in the arteries. If the lipid in the thick intimal tissue of arteriosclerotic arteries were to crystallize, one would expect to find vessels with crystals in their intimas as well as vessels in which the lumina were filled with crystals. Crystals have not been found in the intimas of medium-sized unoccluded arteries. In the similarly sized occluded vessels the crystals were always in the luminal portion of the vessel. This suggests that these large crystals entered the vessels as emboli.

In certain small arteries, however, crystals have been observed in the intimas of vessels in which large lumina were present. It would be dogmatic to say that these lesions were the result of partial recanalization of a vessel previously occluded by cholesterol crystal emboli, although this is a possible explanation. The thickened intimal tissues of these small arteries occasionally contained lipid-filled macrophages. In such vessels cholesterol might have crystallized from this lipid *in situ*.

The possible effects of these vascular occlusions should be emphasized. In case 1, where about 10 per cent of the renal arteries were occluded by these lesions, anatomic changes in the kidneys were produced. These consisted of many wedge-shaped areas of cortical atrophy. In the other cases where medium-sized renal arteries were occluded, similar wedge-shaped areas of cortical atrophy were also found distal to the occluded vessels. No infarcts related to these arterial occlusions have been observed.

It is possible, however, that cholesterol crystal emboli may produce

infarcts in the kidney or spleen, and that gangrene of a toe or some other portion of a lower extremity, occurring in an old person with advanced arteriosclerosis, may occasionally be caused by cholesterol crystal emboli.

Conclusions

Arterial occlusions produced by emboli from eroded aortic atheromatous plaques have been found in the small and medium-sized arteries of the spleen, pancreas, and kidney. In the nine cases in which such lesions were observed the frequency and the distribution of the lesions were variable. In one case the lesions were numerous, involving 19 per cent of the splenic and 11 per cent of the renal arteries; in other cases only a few vessels were involved.

In a typical lesion the lumen of the artery was filled with large cholesterol crystal spaces surrounded by hyperplastic intimal tissue and a few foreign body giant cells. In the kidney these occlusions caused wedge-shaped areas of cortical atrophy.

The intravenous injection of material containing cholesterol crystals, obtained from an atheromatous human aorta, has produced similar lesions in the arteries of the lungs of rabbits.

I wish to thank Mr. Julius Mesiar, who made the photographs which illustrate this paper, and Miss Helen Hirschbein for their technical assistance.

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DESCRIPTION OF PLATES

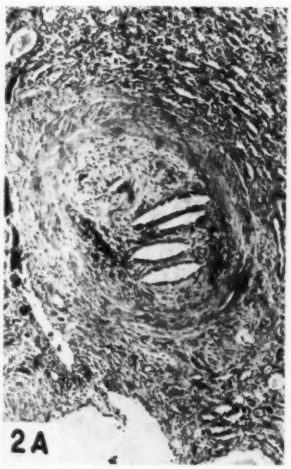
Plate 92

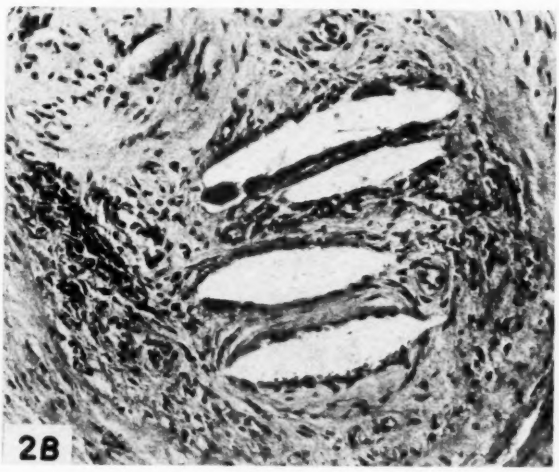
Fig. 1. Case 1. Surface of eroded atheromatous plaque in aorta. The mass of cholesterol crystal spaces is mixed with red blood cells; the surface of this lesion is very rough. × 50.

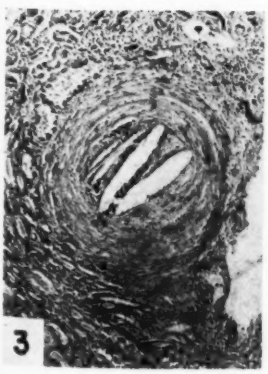
Figs. 2-A and 2-B. Case 1. Occlusion of a medium-sized renal artery. In *Figure 2-A* the entire artery is seen; this measured 880 μ in diameter. The large slit-like spaces in the former lumen of the vessel are cholesterol crystal spaces. × 50. In *Figure 2-B* giant cells can be seen at the ends of some of the crystal-shaped spaces. The dark masses of cells in the left side of the figure are hemosiderin-filled macrophages. × 160.

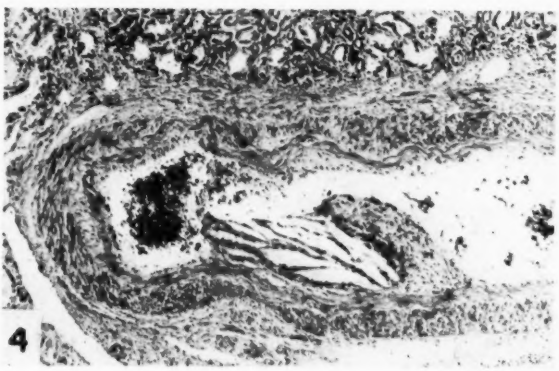
Fig. 3. Case 1. Occluded medium-sized renal artery. In this artery, which measured 825 μ in diameter, the large spaces are cholesterol crystal spaces, the small slits, vascular channels. × 50.

Fig. 4. Case 1. Intimal proliferation about a mass of cholesterol crystal slits in a medium-sized renal artery. In subsequent sections these slit-like spaces fill the lumen of a large branch of this vessel. × 50.







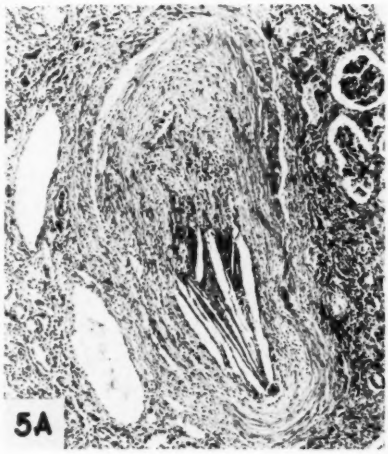


Flory

Emboli from Atheromatous Plaques

PLATE 93

FIG. 5. Case 1. Variability of the pattern of the occlusion in a medium-sized renal artery. In *Figure 5-A* the beginning of the bifurcation of the occluded vessel is shown. Many of the slit-like spaces are cholesterol crystal spaces, others are thin vascular spaces. *Figure 5-B* is the bifurcation of the vessel. In *Figure 5-C* the bifurcation is complete. The branches are partly filled with cholesterol crystal spaces. In *Figure 5-D* the vessels are farther apart. In the upper artery are many small cholesterol crystal spaces; in the lower vessel several large crystal spaces surrounded by giant cells. × 50. In *Figure 5-E* the area of cortical atrophy lying above the vessels shown in Figure 5-D is seen. × 20.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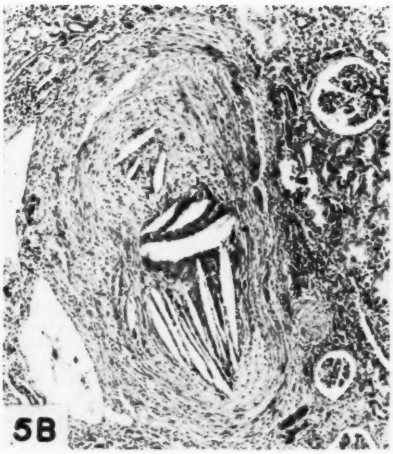
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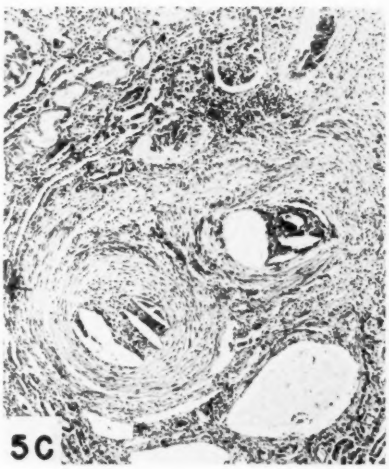
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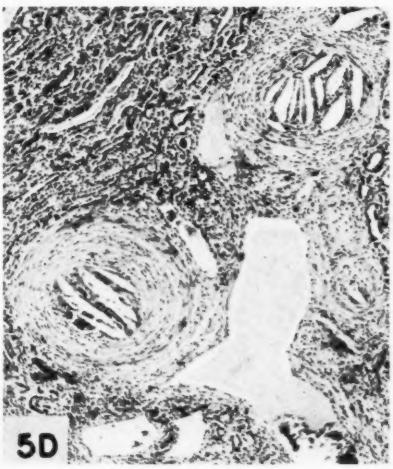
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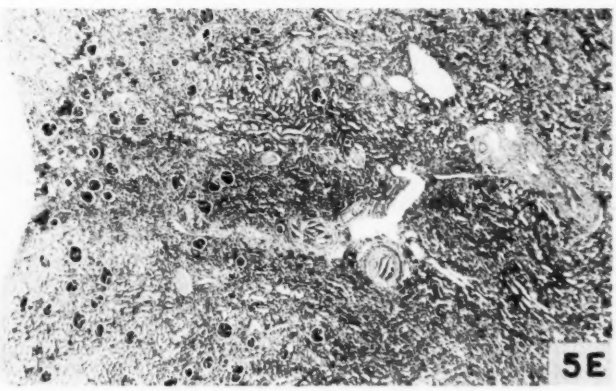

5E

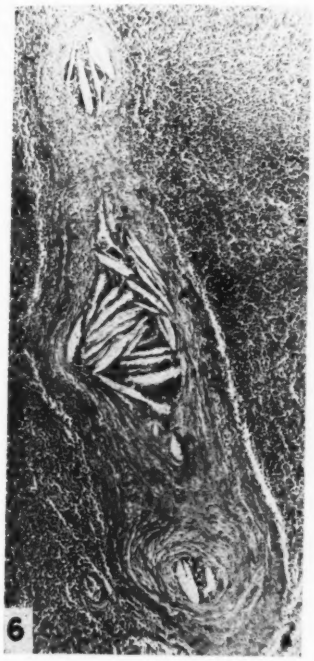
PLATE 94

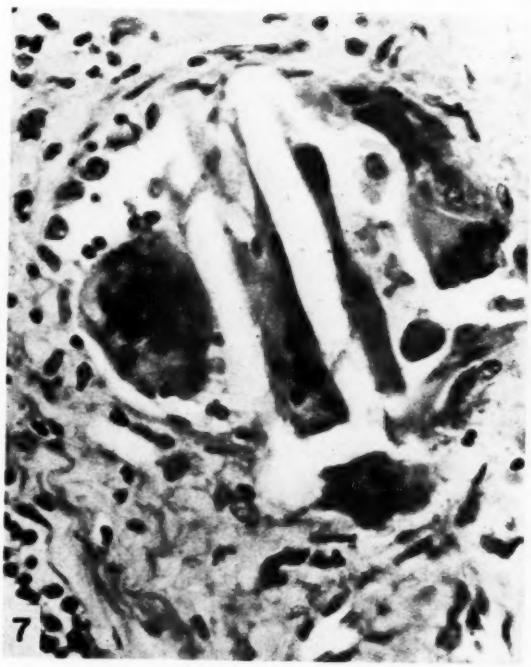
FIG. 6. Case 1. Three occluded splenic arteries. These arteries, the largest of which measured 540 μ in diameter, are probably all branches of a single larger vessel. They are filled with cholesterol crystal spaces. $\times$ 50.

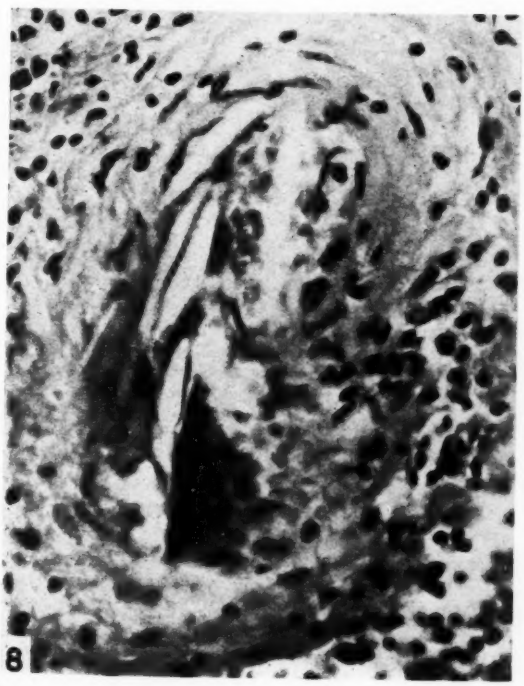
FIG. 7. Case 1. An occluded small splenic artery. This artery is a branch of one of the vessels shown in Figure 6. It measures 94 μ in diameter and is filled with cholesterol crystal spaces surrounded by large foreign body giant cells.

FIG. 8. Case 1. A partially occluded small splenic artery. In this artery, which measured 84 μ in diameter, the cholesterol crystal spaces lie against the intimal surface of one side of the artery and are partly encased by giant cells. Several endothelium-lined vascular channels remain. $\times$ 640.

FIG. 9. Case 1. Partially occluded small splenic artery. The crystal spaces are attached to the surface of the intima of the artery and are almost encased by giant cells. This vessel measures 96 μ in diameter. $\times$ 160.







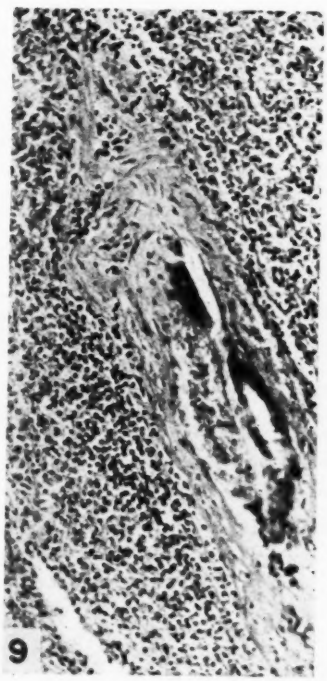


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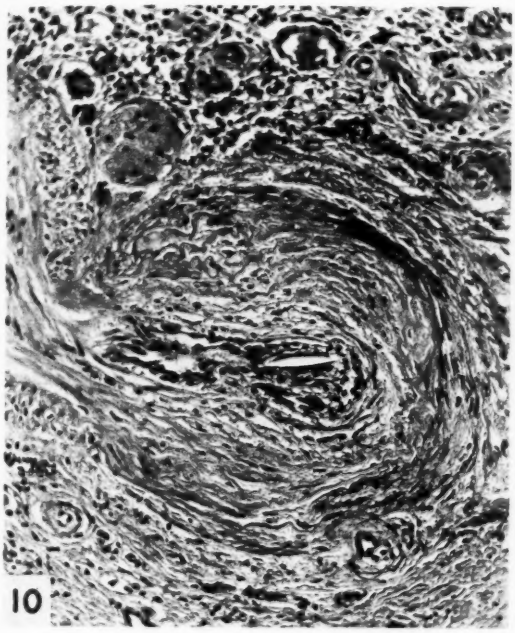
PLATE 95

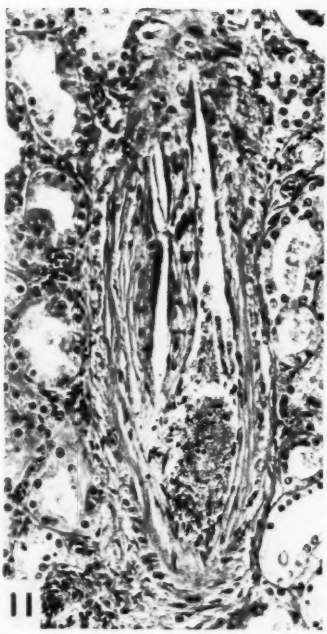
FIG. 10. Case 2. Occluded renal artery. In the center of this vessel are two small cholesterol crystal spaces partly encased by giant cells. Several small vascular channels are present in the intimal tissue. × 160.

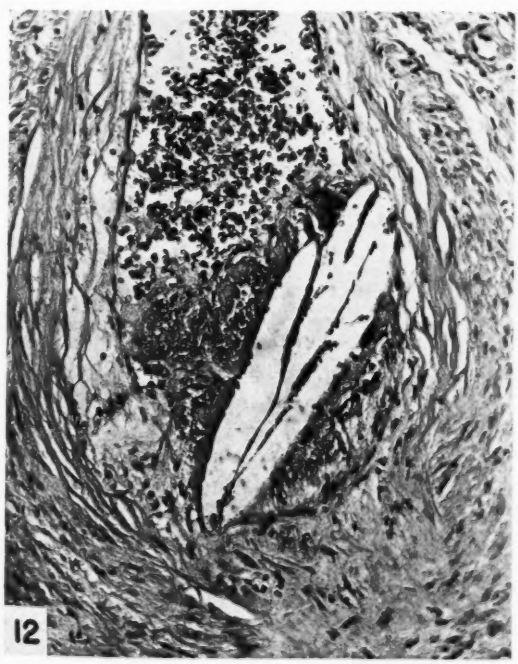
FIG. 11. Case 3. Partially occluded renal artery. Several large cholesterol crystal spaces are embedded in the hyperplastic intimal tissue of one wall of the vessel. A large lumen remains in the artery. This is probably an old lesion, and the vessel is almost completely recanalized. × 160.

FIG. 12. Case 5. Recent thrombus containing cholesterol crystal spaces in a medium-sized renal artery. The lumen of this longitudinally cut artery is occluded or partially occluded by a recent, partially organized thrombus in which are several large cholesterol crystal spaces. × 50.

FIG. 13. Cholesterol crystal space in artery of lung of rabbit. One week before death, this animal was injected intravenously with a suspension of cholesterol crystals from an atheroma of a human aorta. The large crystal space is surrounded by giant cells and intimal tissue. × 640.







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